LANDSCAPE GENETICS AND CWD IN WHITE-TAILED DEER

BY

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DISSERTATION

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ABSTRACT

In this study we used molecular techniques to examine deer movement and population structure in the context of chronic wasting disease transmission and spread. Chronic wasting disease is an infectious prion encephalopathy in cervids that is endemic to Colorado and Wyoming but has spread across the US within the last decade. Quantifying white-tailed deer movement and population structure in infected areas can facilitate predictions of CWD spread via deer dispersal. We analyzed microsatellite genotypes of white-tailed deer populations in southern Wisconsin and Illinois to quantify population level movements, genetic admixture and gender-biased dispersal patterns using F_{ST} and contingency tests. We also examined movements of individuals using assignment tests and spatial autocorrelation, and quantified dispersal events using parentage assignment. Finally, we compared genetic characteristics such as allelic diversity, heterozygosity and fixation indices between CWD infected and uninfected individuals to determine if CWD affects movement of white-tailed deer. Genetic characteristics were not different between CWD infected and uninfected deer, suggesting that changes in movement behaviors associated with clinical illness were not detectable with our molecular data. We found that both male and female deer move extensively in northern Illinois and southern Wisconsin, and that this movement could facilitate CWD spread via dispersal. In contrast, a few locations demonstrated reduced deer movement and female philopatry. One of these locations is a hotspot for CWD in Illinois, and it appears that reduced movements in this area could be exacerbating CWD transmission via direct contact among deer. The observed spatial heterogeneity in deer movement and population structure has important management implications as it allowed us to identify locations at risk for future CWD infection and areas in need of management.

Our study was intented to guide population management and conservation, so we wanted to ensure that biological inferences were based on accurate genetic information. Therefore we

identified sources of genotyping errors, evaluated measures to correct for their presence and provided recommendations to prevent their negative impacts. We detected null alleles in five of 13 previously evaluated microsatellites, and redesigned primers for two of these loci. Analytical corrections for null alleles were unable to fully prevent bias associated with these genotyping errors, and consequently, measures of population differentiation and kinship were negatively impacted. Our results demonstrate the importance of error evaluation during all stages of population studies, and emphasize the need to standardize procedures for genetic marker evaluation.

Since chronic wasting disease management often involves decreasing deer densities to reduce the likelihood of disease occurrence and spread, we wanted to examine the genetic consequences of management in white-tailed deer herds. Increased removal of individuals can alter genetic characteristics of the population, cause a loss of genetic diversity, a decrease in fitness, or enable increased immigration. We compared allele frequencies among cohorts of deer to determine if culling changed the genetic composition of managed populations. Additionally, allele frequency distributions, heterozygosity, and genetic characteristics such as allelic richness and fixation indices were evaluated in pre- and post-cull deer populations to examine the effects of culling on effective population size, genetic differentiation and genetic diversity of white-tailed deer. Cohorts demonstrated little change in allele frequencies from year to year. However, evaluations of pre- and post-cull populations revealed increases in allelic richness and deficiencies in heterozygosity in post-cull populations, suggesting that these populations have received immigrants following intervention. Moreover, female deer, which tend to be philopatric, had significant changes in allele frequencies after culling was initiated. This study suggests that while reducing deer densities through culling enriches the genetic composition of

deer, it could also result in immigration of CWD infected deer, and these potential ecological consequences need to be considered during the implementation of disease management plans.

In this investigation, we also used landscape genetics to examine the effect of landscape features on dispersal and population boundaries of white-tailed deer. An awareness of how the landscape affects animal movement and genetic exchange between populations contributes to our understanding of wildlife ecology. By quantifying genetic structure across the landscape we have identified populations with high and low admixture and discovered gender specific barriers to deer movement that may contribute to CWD spread via dispersal. We found that rivers, streams and interstates contributed to the genetic structuring of females in the study area, but males were insensitive to these features. The observed variations in landscape use between males and females implies that CWD could spread via male movement relatively independently of natural and manmade landscape features, while CWD spread by females would occur over shorter distances because movement is inhibited by these landscape features.

Certain genotypes of the prion gene (*Prnp*) have been shown to prolong disease progression and survival of CWD infected deer. Therefore, examining *Prnp* genotypes in CWD infected and uninfected deer populations can reveal associations between genotype and phenotype to determine if selective pressures are affecting *Prnp* allele frequencies. If selection is occurring, we would expect *Prnp* genotypes that prolong survival to be higher in infected populations compared to uninfected populations. To test this hypothesis, we sequenced *Prnp* of 219 (99 CWD positive and 120 CWD negative) deer from the CWD outbreak region of northern Illinois and southern Wisconsin. We also sampled deer from two uninfected populations: one ~150 km away from the outbreak region, and another ~300 km from the outbreak region. Twelve nucleotide polymorphisms, eight silent and four coding, were found in *Prnp* of the sampled

populations. Five polymorphic loci had significantly different distributions of alleles between infected and uninfected individuals. Nucleotide base changes 60C/T, 285A/C, 286G/A, and 555C/T were observed with higher than expected frequencies in CWD negative animals suggesting disease resistance, while 153C/T was observed more than expected in positive animals, suggesting susceptibility. The total number of polymorphisms per animal, silent or coding, was negatively correlated to disease status. Polymorphisms 243T/A, 286G/A and 555C/T were found at higher than expected frequencies in uninfected populations. The total number of polymorphisms, both silent and coding, also differed between infected and uninfected populations. At the temporal scale examined, selection does not appear to be favoring genotypes associated with CWD resistance as these genotypes tended to have higher frequencies in uninfected populations.

To my family, with your love and support I have accomplished my dreams.
Thanks for ALWAYS being there.

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Chapter 1: Literature Review

A. Illinois White-tailed Deer: Life in a Highly Fragmented Habitat

a. Introduction

For white-tailed deer (*Odocoileus virginianus*), the agricultural landscapes of Illinois are quintessential habitats. Corn and soybean fields provide months of abundant food and cover, while forest-field boundaries and riparian corridors offer plenty of edge habitat (Nixon et al. 1991), a preferred ecotone for deer (Whitehead 1972). Deer can undoubtedly prosper in agricultural environments, and in Illinois this prosperity has led to herd growth, with current estimates approaching 800,000 deer (Illinois Department of Natural Resources 2010). The pervasion of agriculture and urbanization into forested landscapes has resulted in fragmentation of high-quality deer habitats (Nixon *et al.* 1994), especially in the northern part of the state where an increasing human population has exacerbated landscape heterogeneity (Piccolo *et al.* 2001). Resident deer have adapted to fragmentation by taking advantage of forage and cover in row crops during early summer and finding alternative habitats following harvest in late fall (Nixon 1991, Nixon *et al.* 1994). Deer that rely on such ephemeral habitats tend to adopt specialized behaviors and movement patterns that allow them to exploit heterogeneous landscapes (Nixon *et al.* 1991, Nixon *et al.* 1994, Kie *et al.* 2002). In this section of the literature review, we will focus on the ecological characteristics of Illinois deer that have evolved in response to their dynamic environment.

b. Herd History Illinois Deer: Man vs. Wild

In the early part of the nineteenth century, Illinois' prairies and forests were altered dramatically as human settlements cleared continuous stands of forest to make room for fields and pastures. As prairies were replaced and ecotones created, forage and cover for deer improved

because edge habitats were generated and food became more abundant (Calhoun and Loomis 1974). As a result, deer abundance steadily began to rise and eventually peaked in the mid-nineteenth century (Pietsch 1954). Not long after, however, market hunting combined with severe weather and excessive habitat destruction caused a population crash that forced state legislators to ban deer hunting completely (Pietsch 1954, Calhoun and Loomis 1974). Local extinctions seemed to progress from north to south (Pietsch 1954) and by the early 1900's all native populations of deer were considered extinct (Pietsch 1954, Calhoun and Loomis 1974). Though a few wild deer sightings were reported in Southern Illinois around 1910 (Pietsch 1954, Calhoun and Loomis 1974) these animals were believed to have emigrated from Missouri (Calhoun and Loomis 1974), because during this time deer were restricted to a few captive herds and game farms scattered across the state (Pietsch 1954, Calhoun and Loomis 1974). In the early part of the twentieth century, several deer escaped or were deliberately released from these private farms, and it is believed these founder animals contributed to the early stages of herd restoration in Illinois. By the 1940's, the Department of Conservation had initiated repopulation efforts which involved redistributing deer from natural ranges, game farms and refuges into unoccupied habitats across the state. Several hundred deer from the Springfield Game Farm, Rock River range, Emerson Game Farm and Horseshoe Lake Game Refuge were trapped and relocated to Piatt, Cook, Jo Daviess, Pope, Carroll, Alexander, Hardin, and Union Counties just to name a few (Pietsch 1954). Repopulation efforts were extremely successful and resulted in rapid population expansion that has continued for decades (Woolf and Roseberry 1998).

Today, white tailed deer herds are larger than ever (Woolf and Roseberry 1998) and currently, there are few places in Illinois that do not have resident deer. Across the US, deer are becoming so abundant that in certain locales they are considered a nuisance species by some,

especially in urban areas. Vehicular collisions, over-browsing of vegetation, and crop damage can lead to severe economic losses and in some cases loss of human life (Illinois Department of Natural Resources 2010). Unfortunately these types of land use conflicts between humans and deer are predicted to escalate as civilization continues to encroach into wildlife habitats (Bennett *et al.* 2006). Nonetheless, history has proven that deer possess an innate ability to adapt in response to environmental change, and such resilience allows them to continually thrive in Illinois despite ongoing conflicts with the expanding human population.

c. Seasonal Movements

Seasonal migrations are typically initiated during summer months as deer begin to wander away from their home range to forage in nutrient-rich agricultural fields and forests (Nixon *et al.* 1991, Smith 1991). Then in winter, when crops are cleared and high-quality habitats are less abundant, deer reconvene near sources of food and cover, oftentimes in groups of 5-10 (Hawkins and Klimstra 1970, Nixon *et al.* 1991), but sometimes in groups of >30 (Nixon *et al.* 1991, Coulson *et al.* 1997). Seasonal migration in Illinois deer was first documented in 1954, during an observational study conducted near Rockford, IL. Small groups of deer were seen migrating from communal wintering ranges along the Rock River to isolated ranges on woodlots more than five miles away. These outlying ranges were established in April and maintained until late fall when deer rejoined herd members along the river (Pietsch 1954). Seasonal migrations have also been observed in female deer from east-central (Piatt County), northern (Dekalb County), west-central (Brown-Adams Counties) and Chicago, Illinois. In these areas, up to 21.5% of tracked females participated in seasonal migrations, though unlike the deer observed in Rockford, does spent days rather than months on summer ranges and male deer did not migrate (Etter *et al.* 2002, Nixon *et al.* 2008). Migration rates [for this review migration rate=

(# of deer that migrated/total number of tracked deer*100)] for deer in Illinois are considerably less than those observed in other states (Tierson *et al.* 1985, Van Deelen *et al.* 1998), mainly because of variations in habitat. In Michigan for example, migration rates were 40% and 67% for females at separate study sites, both of which were heavily forested (Van Deelen *et al.* 1998). In a heavily forested (>70%) area of New York, 50% of radio-collared deer, both male and female, participated in seasonal migrations (Tierson *et al.* 1985). Like New York, several other states have observed male migratory behavior (Hoskinson and Mech 1976, Tierson *et al.* 1985, Van Deelen *et al.* 1998), though it is rarely documented in Illinois (Pietsch 1954, Etter *et al.* 2002, Nixon *et al.* 2008). Migration rates may be substantially lower in Illinois deer because crops provide ample food and cover during summer months, negating the need for seasonal foraging (Nixon *et al.* 1991, Nixon *et al.* 2008). Further, deer in the northern United States experience extreme winters compared to Illinois, and in these areas, deer need to migrate to find alternate food sources and avoid deep snow (Verme 1973, Nelson and Mech 1984, Tierson *et al.* 1985).

d. Dispersal

Dispersal occurs anytime an individual leaves their home range and establishes a territory in a new habitat. Natal dispersal refers specifically to movements from birthplace to a novel breeding territory, and breeding dispersal refers to movements from one breeding territory to another (Clobert *et al.* 2001). For deer, natal dispersal is often motivated by inbreeding avoidance (Hoelzenbein and Marchinton 1992), aggression from female relatives (Nixon *et al.* 1994), or resource competition (Nixon *et al.* 1991), while breeding dispersal tends to be motivated by mate competition. Interestingly, impetus for movement appears to influence dispersal distance (Long *et al.* 2008). Inbreeding avoidance, for example, can elicit long-distance dispersals in males. Since females are philopatric and tend to share home ranges with relatives

(Hawkins and Klimstra 1970, Long *et al.* 2008), males have to move large distances to gain access to unrelated females outside the matriarchal range. This is in contrast to dispersal motivated by mate competition, where subordinate males only have to move outside the dominant buck's home range to access females (Long *et al.* 2008).

Both natal and breeding dispersal are commonly reported for white-tailed deer in all habitats (Nixon *et al.* 1991, Smith 1991, Nelson 1993, Porter *et al.* 2004), though dispersal tends to be greater in fragmented forests than in larger, more continuous forests (Long *et al.* 2005). For example, dispersal distances in juvenile male deer increased as the proportion of forest cover decreased in 12 deer populations from 8 states. In this study, Long *et al.* (2005) used forest cover as a metric for fragmentation and it accounted for 94% of the variation observed in average dispersal distances for juvenile males. The authors suggested that when resource competition motivates dispersal in fragmented environments, deer must travel longer distances to access food and cover (Nixon *et al.* 1994, Long *et al.* 2005) because habitats are isolated.

Females in fragmented habitats also move more compared to females in continuous forests, but dispersal rates [for this review dispersal rate= (# of deer that dispersed/total number of tracked deer*100)], not dispersal distances are increased (Nixon *et al.* 1991, Nixon *et al.* 1994). For females, dispersal distances were not different between northern, east-central and west-central Illinois populations despite differences in forest cover among habitats (Nixon *et al.* 2007). Dispersal rates, however were higher in east-central and northern Illinois where forests comprise <3% of the land cover, as compared to west-central Illinois which is 20% forested. Only 22% of females dispersed from west-central Illinois, whereas in east-central and northern Illinois almost half of the females dispersed from their natal ranges. Female dispersal rates in west-central Illinois were similar to those observed for females in a 2500 km^2 forest in

Minnesota (Nelson 1993, Nixon *et al.* 2007), where 20% of radio-collared does dispersed in only 3 out of 10 years of observation (Nelson 1993).

In the predominantly agricultural landscapes of Illinois, food is abundant and consequently fawn survival is higher [proportion survived in east-central Illinois: 0.92 (Nixon *et al.* 1991)] than in non-agricultural habitats [eg. proportion survived in southern Illinois: 0.59 (Rohm *et al.* 2007), Texas: 0.75 (Kie and White 1985), Minnesota: 0.31 (Nelson and Mech 1986), and Maine: 0.40 (Long *et al.* 1998)]. With more deer surviving to reproduce, secluded fawning sites can become particularly limited in fragmented environments, and females must disperse to access open niches for rearing young (Nixon *et al.* 1991). Collectively, studies of male and female movement suggest that increased dispersal is an adaptive behavioral trait that allows deer to exploit fragmented habitats with isolated sources of food and cover (Long *et al.* 2005).

Regardless of habitat type, age and gender influence dispersal behavior in white-tailed deer (Nixon *et al.* 1991, Nelson 1993, Nixon *et al.* 1994, Nixon *et al.* 2007, Nixon *et al.* 2008). Male-biased dispersal is commonly observed in deer populations across the country (Hawkins and Klimstra 1970, Nelson and Mech 1984, Tierson *et al.* 1985). In east-central, west-central and northern Illinois for example, male dispersal rates were 57%, 78%, and 68% respectively, while only 49%, 22% and 45% of females dispersed from these same areas (Nixon *et al.* 2007). In addition to gender biases, age also influences dispersal with younger deer, especially yearlings, dispersing from their natal ranges more often than older deer (Nixon *et al.* 1991, Nelson 1993, Nixon *et al.* 1994, Etter *et al.* 2002). While some fawns do disperse, many remain close to their mothers for the first year. Hawkins and Kilmstra (1970) found that in Southern Illinois, male fawns were intimately associated with their mothers for at least one year. Additionally, Nixon *et*

al. (1991) found that approximately half of radio-tracked fawns remained philopatric and spent most of their time socializing with their mothers.

e. Home Range

White-tailed deer in Illinois established larger home ranges in winter than in summer (Nixon *et al.* 1991, Etter *et al.* 2002), and does tended to have smaller home ranges than bucks (Nixon *et al.* 1991). In Piatt County average summer ranges for males were 300 ha, while average winter ranges were ~500 ha. Females in Piatt County also reduced their ranges seasonally with summer ranges averaging 55 ha and winter ranges averaging 177 ha (Nixon *et al.* 1991). Seasonal differences in range size are mainly attributed to availability of resources and reproductive activity (Tufto *et al.* 1996, Said *et al.* 2005). During summer months, deer in Illinois find abundant forage and cover in agricultural fields (Nixon *et al*. 1991), which can eliminate the need for extensive foraging and result in condensed ranges (Nixon *et al*. 1991, Brinkman *et al.* 2005). Female summer ranges are even more reduced during parturition, because females limit their movement to care for fawns, especially within the first few weeks when newborns are too young to accompany their mothers during foraging (Nelson and Woolf 1987, Nixon *et al*. 1991). Unlike females, males begin gradually expanding their home ranges in late summer. Range expansion continues throughout the breeding season when maximum ranges are established and subsequently maintained throughout winter (Nixon *et al.* 1991, Nixon *et al.* 1994). In general, bucks in Illinois choose home ranges based on nutrient availability while does occupy ranges that optimize fawn rearing success. For both genders, site fidelity is high and home ranges are usually maintained throughout their lifetime (Tierson *et al.* 1985, Nixon *et al.* 1991, Nixon *et al.* 1994).

For deer living in fragmented habitats, resources may not be centralized, and linearization of home ranges may be an adaptation to such heterogeneous environments (Halls 1984, Smith 1991, Piccolo *et al.* 2001). In Illinois females in a highly urbanized forest preserve in Des Plaines, IL, occupied linear habitats while does located in a more remote preserve near Palos, IL occupied more circular home ranges (Piccolo *et al.* 2001). Similar findings were reported for deer in Texas where linearity of home ranges was observed for habitats with higher levels of heterogeneity (Inglis *et al.* 1979). Though the study in Texas related home range shape to heterogeneity in vegetation and not to forest fragmentation *per se* (Inglis *et al.* 1979), both studies suggest that landscape patterns influence home range shape.

In addition to altering the shape of their home range, deer have been known to decrease home range size in urbanized habitats. In a suburban community in southern Illinois, winter home ranges for females averaged 37 ha (Cornicelli *et al.* 1996), while home ranges on the outskirts of this metropolitan area averaged 91 ha for females in the winter (Storm *et al.* 2007). These home range sizes are markedly reduced compared to winter ranges for deer in rural habitats in Illinois (177 ha; Nixon *et al.* 1991), New York (132 ha; Tierson *et al.* 1985), and Minnesota (436 ha; Storm *et al.* 2007). These trends were not unique to winter ranges as similar differences in home range size were also observed during summer (Cornicelli *et al.* 1996; Storm *et al.* 2007), and several states, not just Illinois, have reported smaller ranges in suburban areas (Grund *et al.* 2002; Porter *et al.* 2004). It is possible that home ranges are reduced in urban areas because human developments provide an alternate food source for suburban deer (Cornicelli *et al.* 1996; Storm *et al.* 2007). Alternatively, reduction in home range size could be an adaptation to increased density in urban areas, as it would reduce contacts with other deer (Grund *et al.* 2002).

Conclusions

In Illinois, humans and white-tailed deer have shared the landscape for centuries. Our agricultural practices have created a deer utopia that has enhanced their survival and allowed populations to expand. Still, the success of the deer herd in Illinois is only partly attributable to their environment, as their ability to quickly adapt to their surroundings has surely contributed to their persistence. Deer have adapted to the fragmented landscape by increasing dispersal, altering their home ranges, and compared to forested habitats, deer in Illinois are generally non-migratory. If history is an indicator of success, deer will continue to thrive in this man-made environment, and humans will have to balance the benefits of a healthy growing deer herd with the negative consequences of overabundance in some areas.

While extensive information about deer ecology and movement have been realized through observation and radiotelemetry, these studies are limited by sample size, study area, and the difficulties of trapping a random sample (Koenig *et al.* 1996). Given the heterogeneous environment in Illinois and the local adaptations of movement and behavior that have been observed in deer, results from localized studies might not reflect the ecology of the population. Further, local populations are structured by philopatric females, but they are interconnected at a larger scale through male dispersal. This type of regional interconnectivity is indicative of a metapopulation (Weins 1996), though in Illinois, this has yet to be confirmed. Nonetheless, population dynamics among locales are correlated and to ensure effective management, behavior and movement need to be quantified at the metapopulation-level, and thus alternate methods for examining population ecology across a broad geographic scale are needed.

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B. Use of Microsatellite Markers in Population Studies

a. Microsatellites: The Most Valuable Junk in the Genome

In eukaryotic genomes, only one tenth of all genetic material codes for a protein product. The remaining non-coding regions, often referred to as "junk DNA", are not considered true genes by definition, though some are highly functional and a few are well characterized (Moxon and Wills 1999). Introns, for example are non-coding elements interspersed between exons which can manipulate coding regions by modifying RNA splicing sites, inactivating genes (Lewin 2006), or stabilizing chromosome structure (Chistiakov *et al.* 2006). Furthermore, intron length influences transcription efficiency, as genes separated by shorter introns have higher transcription rates than genes with longer introns. For this reason, highly expressed genes tend to be associated with short introns rather than long introns, because primary transcripts and protein products can accumulate faster (Castillo-Davis *et al.* 2002).

The validity of the term "junk DNA" is questionable, yet because few non-coding elements have been functionally described (Moxon and Wills 1999) many still believe they are evolutionary byproducts resulting from random insertions or deletions into ancestral functional genes (Lewin 2006). Despite the lack of apparent function, non-coding DNA is nonetheless useful for tracking ancestral lineages. Since junk DNA does not code for a protein product, evolutionary pressures to maintain nucleotide sequence are oftentimes absent (Lewin 2006), and as a result, these non-coding elements are highly mutable and polymorphic, making them efficient genetic markers (Schlotterer 2000). By far, the most commonly employed genetic markers today are non-coding elements called microsatellites (Oliveira *et al.* 2006). Microsatellites are multi-allelic variable number tandem repeats (VNTR), with each allele corresponding to a different number of repeat units assembled in sequence. Microsatellites

repeats are short, usually between one to six nucleotides (Lewin 2006), and they are dispersed throughout the genome of all living organisms (Li *et al.* 2002). When repeat length is measured for several microsatellites, the individual's allelic profile creates a highly discriminate genetic fingerprint that can be traced back ancestrally, since the alleles are codominant (Chakraborty and Kidd 1991).

Microsatellites were not discovered until the 1980's, though the term "satellite" originated 20 years earlier with the discovery of satellite DNA, a type of repetitive heterochromatin that consists of thousands of repeats. Because of its base composition and compactness, satellite DNA separates from non-repetitive DNA on a density gradient, and the additional band looks like a satellite next to the genomic DNA band. Hence the term satellite DNA was coined, and when much smaller repetitive DNA sequences were later discovered, they were called 'micro' satellites (Ellegren 2004).

Microsatellite mutation rates range from 10^{-6} to 10^{-2} bases per kilobase per generation (Schlotterer 2000), which is 1000 times higher than most functional genes (Chistiakov *et al.* 2006). Strand slippage and homologous recombination are two molecular mechanisms that could effectively introduce mutations at the rates observed in microsatellites (Li *et al.* 2002). Strand slippage occurs during replication when DNA polymerase dissociates from the newly synthesized DNA strand and subsequently reassociates and continues replicating despite misalignment between template and transcript (Fig. 1.1). This process can result in the addition or deletion of nucleotides depending on the location of mismatched repeats in relation to the original replication fork (Schlotterer and Tautz 1992). While strand slippage tends to generate losses or gains of four to10 repeats, homologous recombination can result in vast expansions of up to 200 repeats (Richard and Pâques 2000). This elongation occurs because during

recombination, entire segments of the chromosome are exchanged, sometimes disproportionately, between homologous chromosomes (Li *et al.* 2002).

Even though molecular mechanisms of mutation have been described extensively in the literature, there is an ongoing debate about the forces driving this process. Some research suggests that microsatellite mutation is a random, neutral process while other models suggest that natural selection is involved (Li *et al.* 2002). Evidence for selective neutrality (random, unbiased mutation) comes primarily from models that were able to predict abundance and distribution of microsatellite length based on random walk models (random addition or subtraction of single repeat units) (Bell 1996). These models, however, used a limited number of human and rat microsatellites for validation (Beckman and Weber 1992), and they are not necessarily appropriate for all microsatellites (Li *et al.* 2002).

In fact, recent evidence has shown that the distribution of microsatellite loci is not random and that natural selection plays an important role in regulating their length and position within the genome. In several taxa ranging from plants to primates, mono-, di-, and tetra-nucleotide microsatellites were found at higher frequencies in non-coding DNA than in coding DNA (Wang *et al.* 1994, Metzgar *et al.* 2000, Morgante *et al.* 2002). This biased distribution is believed to have resulted from intense selective pressure to protect against mutations that disrupt the triplet reading frame for codons of functional genes (Li *et al.* 2002). Interestingly tri-and hexa-nucleotide repeats tend to be evenly distributed, or in some cases, at higher frequencies in coding regions of the genome (Wang *et al.* 1994, Morgante *et al.* 2002). Morgante *et al.* (2002) found that in several plant species, frequencies of tri-nucleotide repeats were twice as high in coding regions compared to non-coding regions. Molecular mechanisms involved in protein evolution could be responsible for the inclusion of tri-nucleotide repeats within coding DNA.

Triplet expansion in coding regions creates duplicate codons, and codon reiteration reduces the likelihood that essential amino acids are lost during gene development (Nadir *et al.* 1996).

If neutral models were correct and microsatellites randomly gained repeat units, we would expect to find microsatellite arrays that are very long in some species. There appears to be a threshold for microsatellite length (Garza *et al.* 1995, Nauta and Weissing 1996), however, that implies negative selection against array expansion (Chambers and MacAvoy 2000). This is clearly the case with some microsatellites in humans where repeat expansion is associated with several potentially fatal diseases including Huntington's disease, colorectal cancer, fragile X syndrome and a few neurological disorders (Li *et al.* 2002). Further, microsatellites can regulate gene expression (Martin *et al.* 2005), create hot spots for genetic recombination (Majewski and Ott 2000), and direct telomere synthesis (Chistiakov *et al.* 2006). Therefore, it seems unlikely that all microsatellites were generated randomly given their involvement in genome regulation. Instead, it appears that natural selection regulates the distribution and elongation of many microsatellites within the genome.

Despite the ongoing debate about their origins, in recent years microsatellites have become popular genetic tools in studies of ecology, kinship and evolution (Chapuis and Estoup 2007). Extensive polymorphism and codominant inheritance makes microsatellites ideal for measuring genetic exchange among interbreeding groups or individuals. When applied appropriately, microsatellites provide precise and statistically powerful means of quantifying genetic relationships (Dewoody *et al.* 2006). As a result, hundreds of studies have used microsatellites to examine ecological parameters like parentage (Marshall *et al.* 1998), gene flow (Epps *et al.* 2005, Spear *et al.* 2005), effective population size (Wang 2009), and genetic relatedness (Lynch and Ritland 1999, Lunn *et al.* 2000). These powerful genetic tools have

forever changed the approaches to studying evolution and ecology of species throughout the world.

b. Landscape Genetics Theory

Landscape genetics has been described as a hybridization of landscape ecology and population genetics. The foundation for this emerging discipline was developed in the seventeenth century, when botanists and biogeographers first began to realize that the distribution of organisms is dependent on the physical landscape (Manel *et al.* 2003). Evolutionary pioneers like Alfred Russell Wallace noted that variation in geography and climate of the Malay Archipelago islands coincided with "rich and varied vegetation, their wonderful animal productions", and "the strongly-contrasted races of mankind that inhabit them," (Wallace 1863). Throughout time, interconnections between populations and their environment were further realized, and statistical methods for identifying relationships between genetics and geography began to emerge. Sewall Wright's isolation by distance model, first published in 1943, suggested that in continuously distributed populations with restricted dispersal, interbreeding becomes limited with increasing distance. Wright developed statistics for quantifying genetic admixture based on R.A. Fisher's F-tests, and subsequently defined a set of fixation indices to measure genetic variation among individuals and subpopulations. F_{ST}, for example, describes the level of interbreeding among groups, and is measured as the proportion of genetic variation within a subpopulation relative to variation in the total population. F_{IS}, the inbreeding coefficient, quantifies genetic correlation between gametes, and is measured as the proportion of genetic variation among individuals relative to the variation of the entire population (Wright 1943). Wright's isolation by distance model provided the infrastructure for modern population genetic theory and his statistics are still widely used.

Sewell Wright based his fixation indices on an island model that assumed individuals could randomly breed within a subpopulation (a single island), and subpopulations randomly exchanged migrants at a rate proportional to F_{ST} (F_{ST}=[1/1+4$N_e m$]; N_e = effective population size; *m*= number of migrants exchanged/subpopulation/generation) (Wright 1943, Beerli 1998). Assuming isolation by distance, the proportion of migrants exchanged, and therefore the proportion of genes shared by subpopulations, will be negatively correlated with geographic distance. To test for this statistically, Mantel tests examine relationships between genetic and geographic distance matrices, using random permutations of the observed data to determine significance of the correlation (Mantel 1967, Sokal 1979). While this approach can be useful for preliminary investigations of gene flow, difficulties can arise when randomly breeding subpopulations have to be identified *a priori* (Wright 1943), because genetic admixture oftentimes prevents clear division into groups (Waples and Gaggiotti 2006). Moreover, for some populations, geographic distance alone cannot account for the genetic variation across space, and thus alternate models have been developed to examine genetic variation across the landscape (Raymond and Rousset 1995, Pritchard *et al.* 2000, Peakall and Smouse 2006, Kalinowski *et al.* 2007). In the subsequent section, statistics to quantify genetic patterns and methods to relate these patterns to the landscape will be described. For each method, empirical applications in several species will be discussed, and each technique will be evaluated based on its effectiveness in our sampled white-tailed deer populations.

Indirect Measures of Movement

Dispersal has long been considered the foundation of ecology (Andrewartha and Birch 1954), because it affects the distribution, metapopulation dynamics and genetic viability of the population (Wiens 1996). In the past, movement was measured directly using telemetry, mark-

recapture or observation (Mills 2007), now however, dispersal patterns can be determined indirectly, by measuring gene flow. By comparing allele frequencies among populations, genetic exchange can be quantified and used to infer the amount of movement among habitats (Bohonak and Roderick 2001). While direct measures of movement produce estimates for tracked animals within a recent sampling period, gene flow estimates account for population-level dispersal and migration that has occurred over tens or even hundreds of generations (Bohonak and Roderick 2001). These two techniques can produce different estimates of dispersal, because indirect measures capture the transfer of genes attributable to breeding or individual movements, while direct estimates only document the physical presence of an individual (Scribner *et al.* 2005). Indirect estimates have gained recent popularity because sampling can be easier and more cost effective, thus permitting a large geographic range to be examined (Manel *et al.* 2003).

Contingency Tests

Contingency tests are one way to indirectly determine the level of interbreeding among populations. Even in continuously distributed species, these tests can be applied to delineate populations and measure admixture by comparing allele frequencies among locales (Waples and Gaggiotti 2006). Markov chain Monte Carlo (MCMC) methods (Raymond and Rousset 1995) or random permutation methods (Hudson *et al.* 1992) can be employed to estimate the exact probability that allele frequencies differ between two populations at a single locus. Probabilities for individual loci are combined into a global *P*-value using Fisher's combined probability tests (Fisher 1932), ultimately resulting in a multi-locus evaluation of genetic heterogeneity (Waples and Gaggiotti 2006). Non-significant tests indicate frequent interbreeding among locales and hence a homogenization of allele frequencies across space. Significant tests, on the other hand, suggest that genetic exchange is limited among locales, thereby indicating two populations with

heterogeneous allelic profiles. This heterogeneity can be superimposed over the landscape to identify environmental features that act as barriers to movement (Guillot *et al.* 2009).

Paetkau *et al*. (1998) used contingency tests to examine genetic differentiation in Alaskan brown bears, and they were able to detect six genetically homogeneous populations, one of which was comprised of individuals from multiple coastal islands. Apparently bears frequently swam <2 km between coastal islands to interbreed, resulting in spatial homogeneity of allele frequencies. The authors noted that the contingency tests were extremely sensitive (Paetkau *et al.* 1998), which is why they are effective at delineating populations even with high levels of gene flow (Waples and Gaggiotti 2006). Consequently, this approach was extremely useful for delineating populations of white-tailed deer. Contingency tests were able to detect subtle levels of genetic differentiation resulting from clinal admixture, as well as marked genetic differentiation attributable to manmade barriers (Kelly *et al.* 2010a, submitted).

Assignment Tests

Contingency tests have proven extremely useful for population delineation, but they still require individuals to be allocated into subpopulations *a priori.* This problem can be circumvented with spatially implicit assignment tests that assign individuals to populations based on the distribution of alleles across the entire study area. As with contingency tests, geographic variables can be related to population assignments *post hoc* to identify barriers or corridors for movement. Though these tests can be based on maximum likelihood functions, most assignment procedures used today employ a Bayesian framework for determining an individual's population of origin (Guillot *et al.* 2009). Unlike maximum likelihood, Bayesian statistics incorporate background information from the focal population to simultaneously estimate the probability of multiple interdependent genetic parameters (Beaumont and Rannala 2004). In the program

STRUCTURE (v. 2.3.1; Pritchard *et al.* 2000) for example, Bayesian methods infer the number of genetic clusters (*k)* in the sampled population. STRUCTURE first allocates genotypes into *k* clusters (defined by the user) in a way that minimizes Hardy-Weinburg and linkage disequilibrium, and subsequently calculates allele frequencies for each *k*. Using MCMC (chain lengths can range from 10,000 to 100,000), this process is repeated so that a posterior distribution of allele frequencies is generated for all *k*. Based on this distribution, the likelihood of the model is evaluated by calculating the natural log of the probability [LnP(D)] that individual genotypes belong to their assigned cluster. When this value is compared across multiple simulations with different *k* values, the highest value of LnP(D) is considered the best fit clustering solution for the observed data.

Rosenberg *et al.* (2002) applied Bayesian assignment methods to humans and found that worldwide, there are six genetic clusters located in Africa, southern Asia, eastern Asia, the Americas, Oceania (islands north of Australia), and one distributed across Europe, the Middle East, and Central Asia. Even though the authors sampled at the largest possible spatial scale, they noted that population structure was subtle, and attributable to minute differences in allele frequencies which were better represented as gradual changes across space rather than distinct boundaries (Rosenberg *et al.* 2002). Humans are highly admixed, and low genetic differentiation can prevent population detection when Bayesian methods are employed (Latch *et al.* 2006). Simulation studies have noted that the program BAYESASS (Wilson and Rannala 2003) makes inaccurate assignments when FST<0.05 (Faubet *et al.* 2007), and empirical data shows patterns of isolation by distance can cause Bayesian estimation programs like STRUCTURE (Pritchard *et al.* 2000) and GENELAND (Guillot *et al.* 2005) to overestimate levels of genetic differentiation (Frantz *et al.* 2009).

High gene flow and isolation by distance created problems when STRUCTURE was used to identify populations of white-tailed deer (Kelly *et al.* 2010a, submitted). Because individuals were so admixed, oftentimes equal proportions of their membership were allocated among populations, preventing conclusive determination of their source. Further, replicate runs of STRUCTURE at the same *k* value did not produce the same clustering solution, and at times the distribution of clusters appeared biologically meaningless. Since results from replicate runs were inconsistent, models were ranked based on their Bayesian Deviance value [-2 log LnP(D)], a more stringent criteria for model selection than LnP(D). Bayesian Deviance penalizes for inflations in variance that can occur when MCMC chains become falsely fixated in parameter space, and thus fail to converge (Faubet *et al.* 2007). Faubet *et al.* (2007) recommend running several replicates at different *k* values and selecting the simulation yielding the lowest deviance.

Kelly *et al.* (2010, unpublished) explored selecting *k* based on Evanno *et al.*'s (2005) Δk, (the magnitude of change in LnP(D) between simulations with successive *k* values), which was developed to select the most parsimonious model that best fits the data (Evanno *et al.* 2005). When performing assignment tests of white-tailed deer, Δk promoted parsimony while Bayesian Deviance values seemed to aid in selecting models that converged, but these two methods often selected different clustering solutions. When models were chosen based on Bayesian deviance, genetic differentiation appeared to be overestimated because individuals were not strongly associated with any of the clusters detected. On the other hand, Δk resulted in solutions where individuals were strongly associated with their source populations, yet only one or two clusters were detected across northern Illinois and southern Wisconsin (Kelly *et al.* 2010, unpublished). Collectively these results suggest that immigrants admix substantially with resident deer, thereby preventing conclusive delineations of population boundaries with Bayesian methods.

Spatial Autocorrelation

It is oftentimes necessary to quantify the spatial scale for genetic exchange before meaningful interpretations of population structure can be made (Sokal and Oden 1978). Spatial autocorrelation analysis can be used to examine the spatial interdependency of genetic samples, with positive autocorrelation indicating local homogeneity and negative autocorrelation indicating local heterogeneity (Sokal and Oden 1978). Populations will exhibit positive spatial autocorrelation that declines with increasing distance if gene flow is restricted (Smouse and Peakall 1999), and thus autocorrelation analysis can be used to make inferences about breeding and dispersal.

Global spatial autocorrelation explores spatial structuring across the entire sampled region by examining correlations between geographic and genetic distance matrices at varying spatial scales (Sokal and Oden 1978). With multi-locus data, the genetic distance matrix considered is actually a covariance matrix that describes the tendency for two genotypes to covary in multidimensional genetic space. Covariance values for all individuals within a defined distance class are used to calculate *r*, the spatial autocorrelation coefficient (bounded by -1 and 1) (Smouse and Peakall 1999). To determine statistical significance of the correlation, the observed *r* is compared to a distribution of *r* values generated through random permutation or conditional permutation. Both procedures generate *r* distributions by randomly shuffling samples across spatial locations and subsequently determining the probability of randomly obtaining the observed *r* (Sokal *et al.* 1998). Bootstrapping can also be used to define upper (95^{th} percentile) and lower (25^{th} percentile) confidence limits by randomly resampling *r* values within the specified distance class. When confidence limits do not overlap 0, significant autocorrelation is inferred (Peakall and Smouse 2006).

When spatial structuring is detected on a global scale, it may be because the entire study area is autocorrelated, but it could be reflecting strong autocorrelation limited to a few localities (Sokal *et al.* 1998). Tests for local autocorrelation can detect patterns at a finer resolution than global tests, because *r* is calculated for each individual and its nearest neighbors, rather than for groups within a given distance class (Sokal *et al.* 1998). With local tests, varying numbers of nearest neighbors can be explored to determine how genetic patterns change as more individuals are added to the group. Double *et al.* (2005) used both global and local autocorrelation analyses to describe dispersal at varying spatial scales in superb fairy-wrens. Global tests revealed that female wrens dispersed and male wrens were philopatric, while local tests were able to identify breeding territories with established lineages of dominant males (Double *et al.* 2005).

Spatial autocorrelation was similarly effective in a white-tailed deer population from Illinois and sourthern Wisconsin, as global analysis revealed male and yearling dispersal, and philopatry in adult females and fawns. Local autocorrelation identified habitats that supported large philopatric groups of females, as well as habitats where females dispersal was common (Kelly *et al.* 2010, unpublished). To remain conservative in the interpretation of local autocorrelation, conditional permutation was used for significance testing. While random permutation shuffles genotypes across all sampled locations, conditional permutation fixes the spatial coordinates of the pivotal observation and shuffles the other genotypes randomly. This reduces kurtosis in the simulated distribution and can consequently reduce the type I error rate (Peakall and Smouse 2006). Further, each observed *r* was ranked based on its conditional probability, and then rank values were averaged for each sampled location (Sokal *et al.* 1998). With this method, patterns of spatial autocorrelation at a particular site reflected all sampled individuals rather than a few significant observations.

Detecting Dispersal with Parentage Analysis

For highly mobile species, it is often difficult to discern genetic structure because genes are homogenized across a large geographic range. When gene flow is high, parentage analysis can be useful for dissecting microgeographic structure (Saenz-Agudelo *et al.* 2008) and tracking dispersal (Cullingham *et al.* 2008). The most basic type of parentage analysis relies on Mendelian inheritance patterns of codominant markers to exclude individuals as candidate parents based on genotype mistmatches with the offspring (Jones and Ardren 2003). Ideally, each offspring would be assigned to one parent with the remaining candidates excluded, though this rarely occurs because null alleles and genotyping errors can falsely exclude true parents (Kelly *et al.* 2010b, submitted).

Alternatively, parentage analysis can be based on likelihood ratios, which test the hypothesis that an individual is the offspring's true parent against the null hypothesis that they are unrelated (Jones and Ardren 2003). To calculate a likelihood ratio, genotype matches between candidate parent and offspring are considered, along with the frequency of alleles in the sampled population (the probability of sharing a high frequency allele is higher than the probability of sharing a rare allele, thus the latter contributes more to the likelihood of parentage). The ratios for all candidate parents are log transformed (LOD score), and the individual with the highest value is assigned parentage (Kalinowski *et al.* 2007).

Parentage assignments based on likelihood ratios need to be evaluated statistically to determine if the markers employed can accurately resolve parentage. Programs like CERVUS (Kalinowski *et al.* 2007) use simulations of known parent-offspring pairs to determine LOD values that assign parentage with 95% confidence. To do this, CERVUS first simulates genotypes of 'true' parents (based on allele frequencies of the sampled population), and

genotypes of their offspring by assuming Mendelian inheritance. These parent and offspring genotypes, in addition to randomly generated genotypes of unrelated individuals, are included in a parentage analysis to identify the candidate (either true parent or unrelated) with the highest LOD score for each offspring. Then, LOD scores for true parent assignments are compared to LOD scores for assignments made with unrelated individuals to identify an LOD value that resulted in correct parentage assignment 95% of the time. Extensive overlap between the two LOD distributions indicates that the markers do not have sufficient resolution to assign parentage. However, if the two distributions are sufficiently distinct, then any observed LOD score exceeding the 95% critical value, can be assigned parentage with 95% confidence (Kalinowski *et al.* 2007).

Cullingham *et al.* (2008) employed parentage analysis to determine the frequency and extent of dispersal in raccoons. The authors measured the distance between mother-offspring pairs and found that most individuals travelled >1 km, but a few raccoons dispersed >20 km (Cullingham *et al.* 2008). This example in raccoons demonstrates the utility of parentage analysis in revealing rare, long distance dispersals that are often undetected in studies using direct measures of movement (Koenig *et al.* 1996). This approach proved equally useful in deer population studies, and allowed the identification of dispersals >150 km. However, only 49 individuals were assigned parentage out of 1,991 offspring (Kelly *et al.* 2010, unpublished), most likely because of a lack of genetic resolution from the employed microsatellite panel and the inability to sample the population exhaustively (Jones and Ardren 2003, Kane and King 2009).

Combining Genetic and Environmental Data

Many of the methodologies described thus far involved characterizing genetic patterns and then considering environmental features *post hoc*. Within the last few years, techniques that

use environmental variables directly to explain spatial genetic variation have further advanced the field of landscape genetics (Manel *et al.* 2003). Software packages like BIMR (Faubet and Gaggiotti 2008) perform linear regression with environmental variables to determine which features contribute most to gene flow among populations. Additionally, the program PASSAGE (Rosenberg 2001) conducts partial Mantel tests that incorporate multiple response variables into matrix regression models, thus allowing environmental features to be correlated with genetic distance while accounting for geographic distances between populations (Smouse *et al.* 1986). Using this approach, Blanchong *et al.* (2003) was able to determine that forests with closed canopies promoted movement more so than spatial proximity in white-tailed deer populations from Michigan. Besides population-based regression models, many researchers have adopted individual-based approaches for examining landscape genetics (Coulon *et al.* 2004, Scribner *et al.* 2005, Cushman *et al.* 2006). Cushman *et al.* (2006) performed Mantel tests on individual American black bears and was able to show that forest cover and elevation promoted movement while roads acted as barriers to gene flow. These types of individual-based analyses are extremely useful, especially in highly admixed populations (Latch *et al.* 2006)

Both individual and population-based regression analyses can facilitate the development of least-cost models, which provide insight into how organisms perceive heterogeneous landscapes (Ray 2005). With least-cost modeling, influential environmental variables are considered comprehensively so that the overall difficulty of moving through the landscape can be projected spatially. Effective distances can then be generated from least-cost surfaces to predict the path of least resistance (least-cost path) between two locations (Ray 2005). Least-cost models are not only useful for explaining observed patterns of genetic variation, but they can also predict the importance of potential movement corridors or barriers to gene flow (Cushman *et*

al. 2006). For example, Coulon *et al.* (2004) determined that in roe deer, least-cost distance was a better predictor of genetic distance than Euclidean distance, and from this, they concluded that wooded areas promoted dispersal. In our Illinois white-tailed deer population, BIMR was used to perform linear regression with interstates and rivers as predictors of gene flow. Preliminary investigations revealed that for females but not males, both variables contributed to the distribution of alleles across the landscape (Kelly *et al.* 2010, unpublished), though further analyses are needed to develop a comprehensive least-cost model for deer.

Conclusions

As the field of landscape genetics continues to progress, techniques for quantifying spatial genetic relationships become more advanced and resources for analyzing data more widespread (Manel *et al.* 2003). While the methodologies described above have great potential for discerning spatial genetic patterns, each has limitations and assumptions that must be considered. When estimating gene flow, most methods assume that populations are in equilibrium, such that the effects of genetic drift are offset by the introduction of new alleles as a result of migration Also, genetic markers are assumed to be neutral, or free from selection pressure, so that local differences in allele frequencies can be directly attributed to population genetic structure (Bohonak and Roderick 2001). When assumptions are violated, indirect measures of movement can be inaccurate (Jones and Ardren 2003), and thus it is important to choose methodologies that complement the marker panel (Scribner *et al.* 2005), mobility of the species (Pritchard *et al.* 2000) and sampling scheme employed (Schwartz and McKelvey 2009). Ideally spatial genetic structure should be quantified using several methodologies so that comprehensive biological interpretations can be made (Kelly *et al.* 2010, unpublished).

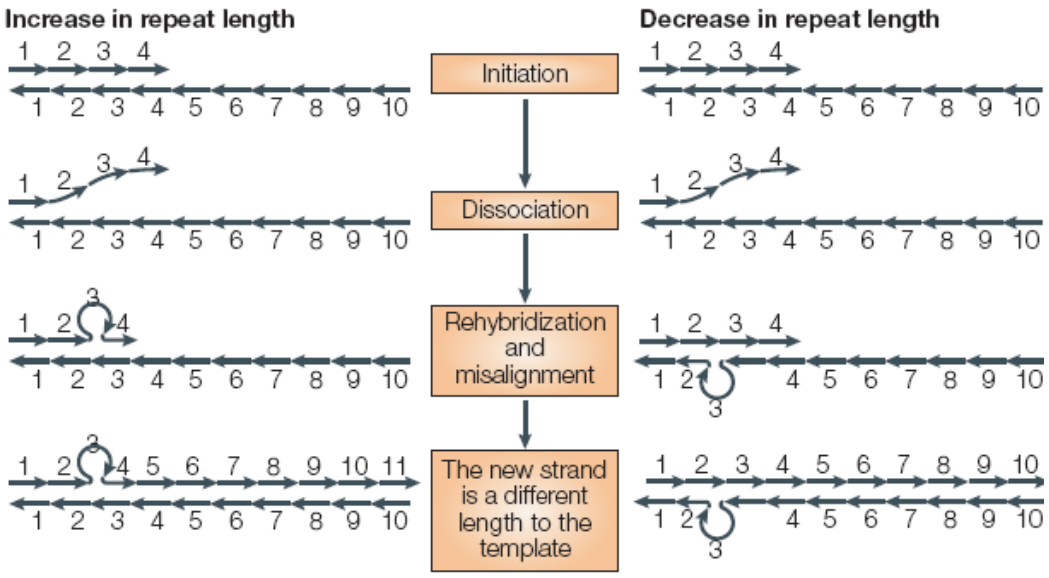

Figure 1.1 Replication slippage. After the replication of a repeat tract has been initiated, the two strands might dissociate. If the nascent strand then realigns out of register, continued replication will lead to a different length from the template strand. If misalignment introduced a loop on the nascent strand, the end result would be an increase in repeat length. A loop that is formed in the template strand leads to a decrease in repeat length.
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C. Chronic Wasting Disease: The Most Transmissible TSE

a. Epidemiology

1. Pathology

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) known to naturally infect white-tailed deer (*Odocoileus virginianus)*, mule deer (*Odocoileus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), moose (*Alces alces shirasi*), and black-tailed deer (*Odocoileus hemionus columbianus*) (Conner *et al.* 2008). As with most TSE, amyloid plaques and large vacuoles in the medulla oblongata are often observed during histological examination of the central nervous system of CWD infected deer (Williams and Young 1980; Spraker *et al.* 1997). The plaques are comprised of neurotoxic (Solforosi *et al.* 2004) aggregates of the abnormal isoform of the prion protein (PrP^{sc}) (Prusiner 1998), and they cause spongiform changes in the brain and spinal cord of CWD infected animals. Post-mortem examination of the nervous system using immunohistochemistry (IHC) is a common method for positive confirmation of TSE because it reveals the deposition of PrP^{sc} in the infected tissues (Williams and Young 1980; Spraker *et al.* 1997; Miller *et al.* 2000; Miller and Williams 2002; Baylis and Goldmann 2004), though ante mortem tonsil biopsies have been used for diagnosis in some cases (Miller and Williams 2002; Wild *et al.* 2002; Wolfe *et al.* 2002; Williams and Miller 2003).

The prion protein (PrP) is an endogenous, membrane-anchored protein that is expressed in neurons, immune cells, and blood cells (Li *et al.* 2001; Burns *et al.* 2003; Novakofski *et al.* 2005). During infection with a TSE, endogenous PrP is converted from a predominantly α-helical secondary structure (47% α-helix) to a predominantly pleated ß-sheet formation (PrP^{sc} ;45% ß-sheet) (Pan *et al.* 1993). Re-folded PrP^{sc} molecules can aggregate tightly, forming fibrils

(Caughey 1993; Prusiner 1998), which provide the necessary interface for further PrP conversion (Prusiner 1998). With suitable intracellular conditions, PrP^{sc} molecules propagate the conversion from protein to protein and then from cell to cell, and finally between systems; PrP^{sc} first spreads through the peripherally exposed tissues to the immune system, and then to the nervous tissues (Sigurdson *et al.* 1999; Miller and Williams 2002; DeJoia *et al.* 2006).

Attempts to recreate the conversion process *ex vivo* have proven that specific intracellular conditions are required to create the infectious isoform of PrP (Jackson *et al.* 1999). Depletion of PrP in cultured neurons can halt further propagation of PrP^{sc} (Mallucci *et al.* 2003), and host-encoded RNA molecules appear necessary to stimulate conversion of PrP to PrP^{sc} *in vitro* and thus may be vital for disease progression (Deleault *et al.* 2003; Liu *et al.* 2007). Additionally *in vivo,* a pool of PrP must be expressed by the host, as PrP knock-out mice do not become infected with prion disease (Büeler *et al.* 1993; Trifilo *et al.* 2007).

In vitro and *in vivo* conversion assays have revealed that the dose required for PrP conversion and subsequent TSE infection can vary widely depending on the PrP^{sc} strain, the route of infection, and the biology of the host (Prusiner 1999). Since most *in vitro* assays were developed in rodents, the kinetics of prion replication in rodent models have been well described and recommended infective doses (ID_{50}) for intracerebral inoculation of PrP^{sc} into hamsters and mice appears to be in the range of $10^{9.5}$-10^{7} units per gram of homogenized brain tissue (Race and Chesebro 1998; Prusiner 1999; Taylor *et al.* 2000). However, ID_{50} as low as $10^{2.9}$ caused subclinical infection when second generation passage of scrapie homogenates were intracerebrally inoculated into mice (Thackray *et al.* 2002). These observations should be considered when interpreting results from interspecies transmission studies, as inferred barriers

to prion infection can be related to ID_{50} used for inoculation more so than true resistance in the host.

2. Intraspecies Transmission of CWD

Though detailed routes of transmission have not been characterized (Sigurdson and Aguzzi 2007), CWD appears to be the most contagious prion disease (Sigurdson 2008). Like scrapie in sheep, direct contact and environmental transmission have been confirmed as modes of CWD transmission (Greig 1940; Hadlow *et al.* 1974; Miller *et al.* 2000; Miller and Williams 2003; Miller and Williams 2004). Oral inoculation of brain homogenate caused CWD in white-tailed deer, moose and cervidized mice (Sigurdson *et al.* 1999; Kreeger *et al.* 2006; Mathiason *et al.* 2006; Trifilo *et al.* 2007), and although urine from CWD infected deer failed to transmit via oral or intracerebral innoculation, blood transfusions, saliva (Mathiason *et al.* 2006) and muscle (Angers *et al.* 2006) have proven to be viable sources of contagious prions following oral and intracerebral innoculation. Consequently, common deer behaviors could facilitate CWD transmission, as saliva can be exchanged when deer mark territories with scrapes and rubs (Kile and Marchinton 1977; Gutschow 2005), visit communal water sources and salt licks (Sigurdson and Aguzzi 2007), or participate in grooming behaviors (Marchinton and Hirth 1984). Further, prions bind to soil particles and remain infectious (Johnson *et al.* 2006), and feces and carcasses have been shown to facilitate environmental transmission in captive herds (Miller *et al.* 2004). Antler velvet could also be an environmental contaminant, as infectious prions were detectable in velvet from CWD infected elk (Angers *et al.* 2009).

Given the number of potential sources for indirect prion exposure and the fact that infectious prions can remain in the environment for >16 years (Georgsson *et al.* 2006), it is not surprising that models of CWD dynamics implicate environmental rather than horizontal

transmission as the main mechanism for disease spread (Miller *et al.* 2006). Also, because deer can have overlapping ranges, exposure would likely be higher for contaminated habitats that support large numbers of deer, implicating a density-dependent model for indirect transmission (Schauber *et al.* 2007).

Alternative to horizontal and environmental transmission, evidence for spontaneous, disease causing mutations in the prion protein (PrP) are known to occur in other species (Collins *et al.* 1999), but they have not been documented in wild populations (Williams *et al.* 2002). To date, vertical transmission has not been conclusively ruled out as a mechanism for spread (Sigurdson and Aguzzi 2007), but it does not appear to be a major source of CWD infection (Miller and Williams 2003).

3. Interspecies Transmission of CWD

If CWD was naturally transmitted to cattle, it would be economically detrimental to the livestock industry (Bunk 2004). Live animals, mouse-models and *in vitro* assays have been used to assess interspecies transmissibility and risk of CWD infection in domestic species. In live animal studies, mule deer CWD was successfully transferred to cattle and sheep via intracerebral inoculation (Hamir *et al.* 2001; Hamir *et al.* 2005; Hamir *et al.* 2006b). Primary intracerebral passages of mule deer CWD in cattle were fairly inefficient though, with less than 50% infectivity and extensive incubation periods (2-5 years). Nonetheless, second generation intracerebral passages resulted in 100% infectivity and a marked decrease in incubation time, indicating that mule deer CWD quickly adapted to bovine hosts (Hamir *et al.* 2006c). Interestingly, cattle were much more susceptible to white-tailed deer CWD, with >85% infection rate following first passage intracerebral inoculation (Hamir *et al.* 2007). In contrast, cattle inoculated orally with mule deer CWD and cattle sharing pastures with infected deer did not

develop disease, suggesting that there is a natural species barrier to prion diseases. Moreover, while oral inoculation caused CWD infection for species within the cervidae family, it has not been successfully transmitted by this route to species in other families, though studies in cattle and sheep are still underway (Sigurdson and Aguzzi 2007).

The presence of infectious prions in various tissues and body fluids raises concerns about interspecies transmission of CWD to wildlife or even humans. Wildlife species that cohabitate with deer are prone to CWD, as voles, mice (Heisey *et al.* 2010) and ferrets (Sigurdson *et al.* 2008) were susceptible via intracerebral inoculation, though raccoons were resistant to infection (Hamir *et al.* 2003). Though CWD has not been documented in caribou, the overlap of CWD outbreaks and wild caribou ranges in Canada is cause for concern. Caribou are commonly consumed in Canada and Alaska and the similarity between caribou and white-tailed deer prion gene sequences suggests that they could be susceptible to CWD as well (Happ *et al.* 2007).

Further, consumption of venison and antler velvet (Bunk 2004) could expose humans to CWD (Angers *et al.* 2006; Angers *et al.* 2009), though it appears that the risk for interspecies transmission is low (Belay *et al.* 2004). CWD exposure resulted in inefficient *in vitro* conversion of human PrP (Raymond *et al.* 2000), and humanized mice did not appear susceptible to elk CWD (Kong *et al.* 2005). Still, studies examining human susceptibility are rare, and many of the early suspect cases of human TSE did not receive autopsies (Sigurdson and Aguzzi 2007). Further, in the late 1990's, three young adults were diagnosed with Creutzfeldt-Jakob disease (CJD). All three had consumed venison or elk during their lifetime, and two of three regularly consumed venison harvested in the CWD endemic area in Colorado and Wyoming. Nonetheless, consumption of CWD infected meat could not be conclusively linked to the source of CJD infection in these patients (Belay *et al.* 2004). Of additional concern, CWD has been documented

in some primate species, as intracerebral and oral inoculation successfully transmitted infection to squirrel monkeys. However, cynomolgus monkeys are more closely related to humans than squirrel monkeys, and they were resistant to both routes of CWD infection (Race *et al.* 2009). To date, the presence of a human species barrier remains unproven.

4. Prion genetics

PrP is highly conserved across mammals. Out of approximately 250 amino acid residues, less than 20 polymorphic sites exist between human, sheep, cattle, and cervid PrP sequences (Novakofski *et al.* 2005). Within the family of cervids, only seven prion protein polymorphisms are known to exist (Raymond *et al.* 2000; O'Rourke *et al.* 1999). Yet, even minute differences in PrP sequence can alter susceptibility to prion disease. In humans, allelic variants coding for methionine at amino acid 129 confer susceptibility to sporadic and variant CJD (Raymond *et al.* 2000). Three PrP alleles that produce protein variants in sheep offer resistance to scrapie, which has allowed domestic breeders to select for resistant genotypes. Mathematical models of scrapie have estimated that breeding resistant genotypes into domestic flocks could reduce current scrapie prevalence from 35% to less than 10% (Gubbins and Roden 2006). In elk, a methionine residue at amino acid 132 (corresponding to human M129V) confers susceptibility to CWD (O'Rourke *et al.* 1999), while 132LL elk appear resistant (Hamir *et al.* 2006a). A number of partially resistant alleles in the prion gene (*Prnp*) were unequally distributed among CWD positive and uninfected white-tailed deer, and protein polymorphisms Q95H and G96S appear extremely protective against CWD in wild deer populations (Johnson *et al.* 2003; O'Rourke *et al.* 2004; Johnson *et al.* 2006; Kelly *et al.* 2008). Recently the G96S allele has been shown to prevent infection from various sources of CWD in transgenic mice (Meade-White *et al.* 2007), and in live animals, deer heterozygous at amino acid 96 showed slower disease progression and

increased incubation periods compared to deer that were homozygous for the wild-type allele (Keane *et al.* 2008).

5. Clinical Symptoms

With continued accumulation of PrP^{sc}, neurotoxic damage progresses and clinical symptoms will eventually manifest. With CWD, hair loss, ataxia, dementia, excessive salivation, polyuria, drooping of the head, and emaciation are commonly cited clinical symptoms (Williams and Young 1980; Spraker *et al.* 1997; Williams and Young 1982). Animals are often affected by these ailments differentially (Williams and Young 1980; Williams and Young 1982; Sigurdson 2008), and in some cases physical symptoms of CWD are subtle or not apparent at all (Williams *et al.* 2002). This was first noted in pathological findings in mule deer where 100% of the animals surveyed experienced emaciation and behavioral changes, but excessive salivation and urination were documented in only about half of all cases (Williams and Young 1980). Studies of CWD-positive elk in captivity demonstrated further symptomatic variation, with only half of CWD infected animals experiencing behavioral changes and excessive salivation (Williams and Young 1992). Interestingly, the first clinical studies of CWD reported grinding of teeth and a fluid filled rumen containing sand and gravel (Williams and Young 1980; Spraker *et al.* 1997), though these symptoms do not appear common with CWD.

Symptomatic variation complicates diagnosis of CWD based on clinical signs in live animals (Williams and Miller 2003), and oftentimes infected deer are asymptomatic during the incubation period. This makes it difficult to identify and remove sick deer from wild populations (Paul Shelton, personal communication), and prevents reliable identification of CWD in farmed herds upon visual inspection (Williams *et al.* 2002). Additionally, even in the absence of clinical symptoms, infected deer may be actively shedding infectious prions and acting as carriers of

CWD (Aguzzi 2006). Orally challenged mule deer fawns were asymptomatic throughout the duration of their illnesses despite the fact that PrP^{SC} was detected in retropahrangeal lymph nodes by 42 days post inoculation. Retropahrangeal lymph nodes could shed PrP^{SC} into saliva, and if so, prions could be transmitted prior to clinical illness (Sigurdson *et al.* 1999)

b. History of CWD

In 1967, researchers studying cervids in a captive facility near Fort Collins, Colorado began to notice behavioral changes in their mule and white-tailed deer. At first it appeared that the deer were depressed, losing appetite and weight, but when fatalities began to occur, it was realized that the condition was much more than an adverse reaction to captivity. The syndrome was termed chronic wasting disease (Williams and Young 1980; Williams and Young 1982; Williams and Young 1992; Miller *et al.* 1998), and at the time the researchers were uncertain of its cause and origin. A decade later, CWD was officially characterized as a cervid TSE based on histopathological spongiform changes in the central nervous system of infected deer (Williams and Young 1980; Williams and Young 1982; Williams and Young 1992; Miller *et al.* 1998; Spraker *et al.* 2002). Between 1967 and 1979, 53 cases of CWD were confirmed in wildlife facilities in Colorado and Wyoming (Williams and Young 1980; Williams and Young 1982), and shortly thereafter, CWD was detected in wild elk (1981) (Spraker *et al.* 2002), wild mule deer (1985) and then in free-ranging white-tailed deer (1990) (Williams and Young 1992; Spraker *et al.* 1997; Miller *et al.* 1998).

During the 1990's, CWD seemed to remain endemic to the western United States, but increased surveillance efforts initiated by CWD management teams across the country revealed that this disease was not geographically contained. In 1997, CWD surfaced in a captive herd of elk in South Dakota and within the next few years, it was discovered in captive herds in

Montanta and Oklahoma and in free ranging populations in New Mexico and Utah. In Kansas and Nebraska, CWD was detected in both captive and wild cervids (Williams *et al.* 2002; Joly *et al.* 2003; Salman 2003; United States Geological Survey 2010). To the surprise of many, multiple outbreaks soon appeared east of the Mississippi in 2002, when a herd of white-tailed deer in Wisconsin tested positive for the disease, and shortly thereafter CWD was found in Illinois and Minnesota (United States Geological Survey 2010).

The sporadic pattern of CWD outbreaks spanning the U.S. (Sigurdson 2008) suggests that deer movement is not solely responsible for disease spread. Though CWD seems to spread through animal dispersal in endemic areas (Conner and Miller 2004), most experts believe that transportation of infected, captive cervids from endemic areas caused the geographic expansion of CWD across the U.S. and into other countries (Williams and Young 1992; Williams *et al.* 2002; Joly *et al.* 2003). The first case of CWD in Saskatchewan, Canada for example, has been linked to a farmed herd in South Dakota, and importation of CWD positive elk from Canada has been cited as the source of infection in South Korea (Sohn *et al.* 2002; Williams *et al.* 2002; Kahn *et al.* 2004). The majority of U.S. states with CWD have found infection in both captive and wild populations (Sigurdson 2008), most likely because geographic overlap between cervid farms and free-ranging deer habitat increases the risk of intraspecies transmission (Williams *et al.* 2002; Joly *et al.* 2003). Recently, CWD was detected in captive white-tailed deer herds in Missouri and free-ranging mule deer in North Dakota. As of March 2010, United States Geological Survey reported CWD in 17 states, 2 Canadian Provinces and multiple imported cases in South Korea (Sohn *et al.* 2002; Kim *et al.* 2005; United States Geological Survey 2010).

The source of infectious prions in captive facilities remains unknown, though several hypotheses have been suggested (Williams *et al.* 2002; Salman 2003). One popular explanation

names scrapie as the original TSE agent that gave rise to CWD (Spraker *et al.* 1997; Race *et al.* 2002; Salman 2003), and coincidentally, sheep studies were conducted in the same research facilities where CWD was discovered (Williams and Young 1980). The scrapie hypothesis is further supported by the fact that glycoform patterns of the two TSE are nearly indistinguishable (Race *et al.* 2002), and CWD brain homogenate can facilitate conversion of *in vitro* sheep PrP into PrP^{sc} experimentally (Raymond *et al.* 2000), and both TSE share the ability to persist in populations, mainly through horizontal transmission (Race *et al.* 2002; Williams 2003). Yet, despite similarities between the two TSW, scrapie is limited to domesticated animals and CWD is not, and countries like the United Kingdom have maintained a higher prevalence of scrapie and exhibit no evidence of CWD (Spraker *et al.* 1997). Additionally, incubation periods for CWD inoculated sheep are long (Hamir *et al.* 2003), and low conversion rates of PrP to PrP^{sc} with a CWD catalyst (Raymond *et al.* 2000) suggest that natural cross-species transmission between cervids and ovids is not favored, counterintuitive to the scrapie hypothesis.

Alternatively, a spontaneous mutation in cervid PrP could have given rise to an infectious isoform that was subsequently spread to other deer and elk (Spraker *et al.* 1997; Williams *et al.* 2002). Other prion diseases, such as spontaneous CJD in humans (Gajdusek 1996), are transmissible, but to date, no sporadic occurrences of TSE have ever been documented in wildlife populations (Williams *et al.* 2002). It is also possible that an unidentified TSE is present in a wildlife reservoir (Salman 2003; Sigurdson 2008; Heisey *et al.* 2010), or CWD could have gone undetected in wild cervid populations before it was found in captive deer (Miller *et al.* 2000), which implies a spillback rather than a spillover from captive to wild deer. Unfortunately, most experts agree that it would be nearly impossible to conclusively trace the origins of CWD back to either wild or farmed cervids (Spraker *et al.* 1997; Williams *et al.* 2002), and for now the

consensus is that the epicenter resides in north-central Colorado and south-eastern Wyoming (based on contemporary disease prevalence and current distributions) (Miller *et al.* 2000).

c. CWD Management

To date, there are no treatments for CWD, so control measures revolve around disease surveillance, herd depopulation, and cervid transportation bans to monitor and prevent disease (Salman 2003). Population reductions can be targeted towards infected individuals or randomly employed to reduce cervid densities, but to be successful, they must reduce further transmission to contain CWD (Gross and Miller 2001; Conner *et al.* 2008). In parts of New Mexico and in Estes Park, Colorado, deer were radio-collared and routinely tested for CWD using tonsil biopsies, then when CWD was detected, infected deer were located and subsequently euthanized (Wolfe *et al.* 2004; Mower 2005). However, the estimated cost of this management tactic was $300 per deer, and simulation studies modeling the efficacy of this approach suggest that at least 20% of the infected animals must be removed to eventually eliminate CWD from the herd (Gross and Miller 2001). Hence, while this approach may be useful for small herds or enclosed areas, large-scale eradication would not be possible with test and cull management (Wolfe *et al.* 2004).

In the CWD endemic areas of Colorado and Wyoming, disease eradication is not feasible because the outbreak spans >80,000 km^2. Therefore in Colorado and many other states, culling is directed at deer within close proximity to infected locations, using either trained sharpshooters or open-access hunts (Conner *et al.* 2007), and CWD containment efforts are focused on habitats that promote deer movement (Conner *et al.* 2008). In 2007, Conner *et al.* found that herd depopulation may not be effective, as areas of Colorado that received directed culling treatments did not see a reduction in CWD prevalence compared to areas that were not managed. However in this study, directed culling was conservative, with fewer than 20 deer harvested from large

management areas that averaged ~100 km^2. Thus, the number of infected animals removed might have been insufficient to decrease disease, or the temporal scale was too short to see significant changes in prevalence (Conner *et al.* 2007). Unfortunately, most CWD management programs have only been in practice for a few years and as a result, studies that evaluate control strategies are rare. To effectively discern the effects of CWD management, additional data collected over a larger temporal scale will be required to document changes in disease prevalence.

d. CWD in Wisconsin

In Wisconsin, the search for CWD in free-ranging white-tailed deer populations began in 1999 when the Wisconsin Department of Natural Resources (WDNR) tested >1,000 hunter harvested deer for prion disease. Though surveillance failed to detect positive cases during the first three years of testing, in 2002, three adult bucks shot during the 2001 fall hunting season in southwestern Wisconsin confirmed that CWD had officially spread into the Midwest (Joly *et al.* 2003). Management agencies reacted immediately by placing state-wide bans on cervid importation, baiting and feeding wild cervids, and the use of salt and mineral licks. During 2002, WDNR identified core areas for targeted management based on their proximity to infected locations. The eradication zone included areas surrounding the first cases of CWD in Wisconsin (up to ~10 mi), and was considered the area of highest CWD risk. In the eradication zone, hunting seasons were extended and additional permits were issued to landowners and WDNR employees in an effort to remove as many deer as possible. WDNR also designated a 450 square-mile area surrounded the eradication zone where hunting regulations were liberal and extensive CWD testing was conducted to determine the full extent of the outbreak (Wisconsin Department of Natural Resources 2002).

At first, the defined management zones appeared to encompass the disease epicenter, however, during fall hunting season 2002, CWD positive deer were found in southeastern Wisconsin and also in northern Illinois, indicative of another disease focus. During the first year of intense surveillance and CWD management, Wisconsin detected 205 cases of CWD. The management zones established in 2002 were enlarged between 2003 and 2006 to keep up with the expanding outbreak, and by 2007, 19 counties in southern Wisconsin were considered part of the CWD management zone. Approximately 100-200 cases have been detected in this area every year since 2002, and to date, 1,345 Wisconsin deer have tested positive for CWD (Wisconsin Department of Natural Resources 2010).

The CWD outbreak in southwest Wisconsin (Mt. Horeb) is distributed heterogeneously across space (Osnas *et al.* 2009). Prevalence is highest near the epicenter, and decreases with increasing distance from the core (Joly *et al.* 2006). The Wisconsin River seemingly acts as a northern barrier to disease spread, as it deters movement in resident deer (Blanchong *et al.* 2008). Heterogeneity of infection is also reported among demographic groups, with male deer having a higher probability of infection than females, and older deer having a higher probability than younger deer. Older deer are more prone to CWD because they have had more time to be exposed, and increased direct contact during breeding seemingly increases exposure in polygamous males compared to females (Grear *et al.* 2006; Osnas *et al.* 2009). In Wisconsin, research on CWD continues to be focused towards modeling disease dynamics and understanding spread and transmission. Scientific insight is needed not only to evaluate past management actions, but also to guide future CWD management programs in wild deer populations (Wisconsin Department of Natural Resources 2010).

Overall, the fight to control CWD in Wisconsin has proven extremely difficult and expensive. Between 2002 and 2006, the state spent an estimated $25 million on CWD management (Unger 2007), with an estimated $68 million lost each year in hunting revenue (Bishop 2004). This loss is mostly attributable to a dramatic decline in hunting license sales after CWD was discovered (Heberlein 2004). To the dismay of managers, models of CWD have shown that prevalence is increasing in many areas of Wisconsin. This increase, in addition to declining public and political support, has forced WDNR to abandon the concept of disease eradication in favor of strategies that focus on preventing further geographic spread. Despite the increase in CWD prevalence, the Wisconsin deer population appears to be declining (Wisconsin Department of Natural Resources 2010), suggesting that deer reduction efforts have been effective. Nonetheless, it is too soon to fully understand the ecological impacts of Wisconsin's disease management strategies, and future research will be required to determine if CWD spread has been successfully minimized.

e. CWD in Illinois

The first case of CWD in Illinois was confirmed on November 1, 2002 in a free-ranging deer that was demonstrating clinical symptoms. Between 2002 and 2003, over 4,000 hunter-harvested samples had been tested through routine surveillance programs that were initiated several years prior. As soon as CWD was confirmed, the Illinois Department of Natural Resources (IDNR) began using surveillance to collect additional samples for testing so that the distribution of disease could be determined. In 2003, experimental herd reductions efforts were initialized to control CWD. Unlike Wisconsin where depopulation efforts are spread over a large management zone, IDNR deer population control targeted areas immediately adjacent to CWD infected locales to remove sick deer and reduce population densities. With the various surveillance

methods employed in the first year, fifteen CWD infected deer were found in northern Illinois, with nine cases from Boone County, four cases in Winnebago County and two in McHenry County(Illinois Department of Natural Resources 2010).

By late 2003, the CWD outbreak in Illinois appeared to spread along riparian corridors into DeKalb County. However, the prevalence for most infected areas was less than 5%, so it was difficult to determine if CWD spread or was previously undetected in DeKalb County. Consistent surveillance in the outbreak region of Illinois began to show evidence for three main foci of CWD: one to the southeast of Rockford**,** one to the northeast of Rockford, and one in northeast DeKalb County. Nonetheless, in 2005 the distribution expanded as CWD was detected in a deer from western Winnebago County and in 2006, two CWD positive deer were found in Ogle County, almost 30 miles from the original disease foci. These cases appeared to be isolated rather than samples from established outbreaks, because extensive testing in subsequent years detected only one other CWD positive deer in these areas. Likewise, the CWD positive deer found in 2008 in LaSalle County appeared isolated, as it has been the only case detected south of DeKalb County in seven years of surveillance (Illinois Department of Natural Resources 2010).

Compared to the outbreak near Mt. Horeb, Wisconsin, the spatial distribution of CWD in Illinois is patchy. CWD prevalence in the three foci can be as high as 10%, but in some of the adjacent townships, prevalence is zero. The sporadic distribution of CWD in Illinois makes targeted management difficult, thus aerial surveys are used to determine deer density and identify over-wintering refuges near CWD infected locations that could harbor infected deer. This method allows for concentrated surveillance and disease management efforts in high risk areas which is important considering that geographic expansion of CWD has increased the demands for wildlife managers. It appears that in Illinois, targeted culling has been successful at removing

sick individuals from the herd. During 2002-2009, IDNR sharpshooting accounted for 37% of positives identified statewide, although only 12.4% of tested samples were collected through agency sharpshooting (Illinois Department of Natural Resources 2010). Further, preliminary evaluations of CWD control strategies in Illinois show declines in both deer densities (Shelton *et al.* 2010) and disease prevalence for culled areas (Weng *et al.* 2010, in preparation), an encouraging prospect for future disease management.

Conclusions

While it appears that CWD management has been effective in Illinois, many other states have not observed positive outcomes from disease control, and thus, many managers have adopted a bleak outlook on CWD eradication. In 2006, the Colorado Division of Wildlife announced that they would stop random culling because their research showed "no clear evidence of any kind of beneficial affect" (Mike Miller, Colorado Division of Wildlife veterinarian; Woodward 2007). Wisconsin and many other states face growing public opposition to herd culling and budget reductions for management programs (Wisconsin Department of Natural Resources 2010). Hunters in Wisconsin are concerned that the WDNR's management program is too invasive and they blame a decrease in harvest success on the state's herd reduction plan (Wisconsin CWD Watch 2010). Many people that oppose Wisconsin's management efforts are quick to point out that CWD has not been detected to humans, but they are failing to realize that human studies are rare, and even the experts warn against making hasty conclusions about potential species barriers (Belay *et al.* 2004). Mad-cow disease was originally thought to be harmless to humans and "eating beef and drinking milk from British cows" was considered safe according to the United Kingdom's Ministry of Agriculture, Fishing and Food in 1996 (Jacob and Hellström 2000). We realize now that British officials were wrong, and 138 human lives and an estimated ten billion

dollars (Jacob and Hellström 2000) have been lost as a result. CWD is not fully understood, and if anything is to be learned from the BSE epidemic, we should avoid making assumptions about the potential zoonotic transmission of CWD to humans until we have more information about prion disease dynamics.

In wild deer populations, CWD epidemics are capable of reducing survival and population viability (Miller *et al.* 2008; Edmunds 2008). Since deer are keystone herbivores (Waller and Alverson 1997), the impacts of CWD could presumably alter entire ecosystems, especially without management intervention (Miller *et al.* 2008; Gross and Miller 2001). Hence, despite opposition, CWD management and research are still warranted until the consequences of CWD in wildlife, livestock, and humans can be fully understood.

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D. Management of White-tailed deer

In North America, white-tailed deer have been recognized as a natural resource for hundreds of years, first by Native Americans, then by European settlers, and now by modern American society (Langenau Jr. *et al.* 1984; Cote *et al.* 2004). Deer are important ecologically, because as herbivores, they are capable of shifting local flora, which can drastically alter natural communities (Alverson *et al.* 1988; Diefenbach and Palmer 1997; Waller and Alverson 1997). Deer are also a source of food, wildlife recreation and income, and so federal and state agencies have fought to preserve herd health and stability for over a century (Fagerstone and Clay 1997).

In the United States, the white-tailed deer population has proven extremely dynamic, and over time, deer managers have had to modify population control strategies to adapt to oscillating densities (Decker and Connelly 1989). Deer managers in the early part of the twentieth century sought to create edge habitat and limit hunting to stimulate population growth, but now, most herds require reductions to prevent deer from exhausting their resources (Smith and Coggin 1984; Cote *et al.* 2004). In this section of the literature review, we will discuss traditional deer management practices as well as recent advances in the field that have been developed to aid wildlife management in urban settings.

a. Hunting Regulations

White-tailed deer are the most popular big-game species in North America (Boone and Crockett Club 2006), and so it is not surprising that hunting and wildlife recreation generate billions of dollars in revenue each year (Opsahl 2003). Sustaining viable herds has always been a management priority, not only because it contributes the conservation of wildlife ecosystems, but also because continued interest in wildlife recreation promotes an awareness of natural resources as well as economic returns (Fagerstone and Clay 1997). Hunting is oftentimes required to

maintain a stable deer herd, because it helps balance reproduction and mortality. Hence, deer managers manipulate deer mortality by allowing liberal harvests when populations are abundant, or enforcing strict hunting regulations when populations decline (Hayne 1984; Brown *et al.* 2000). For example, when the goal is herd reduction, managers may choose to permit harvest of both genders, lengthen the hunting season or increase the number of hunters. When the goal is to increase abundance, managers can enforce a buck only harvest, reduce the number of permits issued, or limit the type of weapon that can be used (Smith and Coggin 1984).

When federal agencies began regulating deer populations in the nineteenth century, their goal was to reduce mortalities from habitat loss and market hunting (Decker and Connelly 1989). During this time, hunting was completely banned in some public areas and many private properties (Diefenbach and Palmer 1997), and in several states, buck laws prohibiting doe harvest were passed (Leopold *et al.* 1947; Brown *et al.* 2000) to stimulate reproduction and herd growth (Leopold and Arms 1931). In 1937, the Federal Aid in Wildlife Restoration Act was implemented to protect fish, big game and their habitats by recirculating tax money generated from fishing and hunting into state agency restoration programs (Boone and Crockett Club 2006). To date, this program has supported hundreds of wildlife research projects and assisted in the development of wildlife habitats across the country (Smith and Coggin 1984).

By the mid 1900's, deer populations had rebounded, and 47 states supported one or several herds. In fact, states like Arizona, Maine and Wisconsin reported problems with starvation and overbrowsing in a few isolated herds prior to the 1950's. However, this localized overabundance was mostly attributed to ineffective management strategies at the time (Leopold *et al.* 1947). Except in the southwestern US where deer herds had not yet approached carrying capacity, deer in many states were able to reproduce at a rate that was higher than their mortality

rate, which meant that a surplus of individuals could be readily harvested each year without causing permanent reductions in density. Wildlife managers began legalizing antlerless harvests to stabilize population growth (Leopold *et al.* 1947; Brown *et al.* 2000), and eventually, many state agencies adopted a quota system that regulated the number of hunting permits issued based on the estimated surplus of deer in the area (Decker and Connelly 1989).

During the mid-twentieth century, research in wildlife management gained momentum (Boone and Crockett Club 2006). Populations within the George Reserve in Michigan provided opportunities to study deer in enclosed habitat (McCullough 1984), while places like the Crab Orchard National Wildlife Refuge in southern Illinois were valuable for monitoring herd dynamics in natural settings (Roseberry and Woolf 1991). With these "outdoor laboratories," biologists were able to experimentally examine population processes like reproduction, mortality and recruitment (McCullough 1984), which would later provide the framework for contemporary models of harvest management (deCalesta and Stout 1997).

Demographic data collected from deer harvests and road kills provide the input for most analyses concerning population trends (Roseberry and Woolf 1991). Mortality, for example, can be inferred by tracking harvests for a single cohort over time, and reproduction rate can be measured by counting embryos in harvested does (Hayne 1984). Estimates of recruitment can be obtained from fawn:doe ratios in road killed or harvested deer (Roseberry and Woolf 1991), and abundance can be determined by directly observing the herd, counting fecal deposits, conducting surveys of dead deer, or examining harvest success (Hayne 1984).

Roseberry and Woolf (1991) reviewed several analytical methods to infer abundance and age structure from harvest data collected at the Crab Orchard National Wildlife Refuge. Their findings suggested that models considering the number of deer harvested in relation to hunting

effort (number of hunters or permits issued) seemed to produce reasonable estimates of pre-hunt population size. The authors also recommended summing mortality for a single cohort in subsequent years of harvest to estimate survival for a particular age group, a method known as population reconstruction (Roseberry and Woolf 1991). The findings of Roseberry and Woolf (1991) underscore the importance of using multiple approaches to estimate population size, as each method they evaluated had specific assumptions, advantages and limitations. Further, managers have to consider the bias of available data and the appropriateness and accuracy of methods employed to estimate population processes (Hayne 1984).

Quantifying characteristics like mortality, abundance and recruitment is important because these variables are incorporated into models that predict the population's response to hunting and estimate the harvestable proportion of the herd (Halls 1984).With these estimates, deer managers can determine sustainable yield, or the number of deer that can be harvested annually without causing irreversible reductions in density. Sustainable yield is dependent on management objectives, as densities that are acceptable for farmers experiencing crop damage may be too low for hunters. Hence, harvest regulation can be a subjective process at times, as it requires consideration of social demands as well as scientific insight (Roseberry and Woolf 1991).

b. Habitat Management

Although deer are habitat generalists, their nutritional and ecological requirements are better met in habitats that offer a variety of ground covering plant species for forage (Harlow 1984). Floral diversity is important for deer because plants that provide high quality forage are not always optimal for shelter and cover. This is one reason deer prefer edges habitats, these areas are

diverse and offer cover in closed canopy forests adjacent to clearings that provide food (Fulbright and Ortega-S 2006).

By understanding vegetational preferences of the herd, managers can manipulate environment conditions to improve habitat quality, or if needed introduce unfavorable habitats to keep densities low (Alverson *et al.* 1988). Prescribed burns are one way that managers can promote the growth of early succession plant species, which provide high quality forage for deer. In Tennessee and Florida, increases in nutritional quality and quantity of deer forage were reported in burned habitats (Dills 1970; Carlson *et al.* 1993), and in Missouri, burns provided deer with open habitats that contained a wide range of plant species (Crawford 1984).

Managers can further improve deer habitat by clearing sections of forests to provide openings for early succession plants (Alverson *et al.* 1988). Mowing, plowing and herbicides have been used to reduce the amount of closed canopy in an area or remove nutritionally inferior plants. Clearing promotes floral diversity, and creates edge habitats for deer (Fulbright and Ortega-S 2006). This has proven to be an effective way to increase preferred forage and deer abundance in Minnesota. In states like Arkansas, Delaware and Indiana, wildlife agencies regularly incorporate forest clearing into deer management plans (Crawford 1984). In contrast, preserving large blocks of mature, late succession forests has been proposed as a way to reduce local densities by redistributing deer into alternate habitats (Alverson *et al.* 1988), though the effectiveness of this strategy has not been thoroughly evaluated. All of the methods for reducing continuous stands of forests are intrusive to the environment, therefore they must be carefully implemented to provide the desired outcome. Also, clearing will eventually result in regrowth, thus continued habitat management must be applied to ensure that forage quality is sustained over time (Alverson *et al.* 1988).

Planting food supplements is another way that managers can increase herd health and abundance, though this technique has also been used on private lands to attract deer for harvest. In Texas, one study showed that 56% of surveyed landowners plant some form of food plot for white-tailed deer (Adams *et al.* 1992). In the Midwest, agricultural fields are not planted to provide wildlife food supplements, but they have nonetheless led to high survival because crops offer plentiful food and cover for deer (Nixon 1991). In non-agricultural habitats or during winter months, clover, honeysuckle, and conifers can be planted to supplement limited resources. Supplements improve nutritional quality of food, which led to an increase in body mass and antler quality for deer in Mississippi (Fulbright and Ortega-S 2006), and increases in weight of young male deer in Louisiana (Keegan *et al.* 1989). However, not everyone agrees that planting is beneficial, not only because it is expensive and temporary (Fulbright and Ortega-S 2006), but also because it can attract unwanted wildlife and facilitate transmission of diseases like bovine tuberculosis (Miller *et al.* 2003).

It is difficult to determine if habitat manipulation is an effective way to manage deer because studies that track improvements in herd health following prescribed environmental alterations are not common. This is an area that warrants future research because the ecological consequences of changing the composition of the landscape are at times severe, and they can impact several species across multiple trophic levels (Crawford 1984; Fulbright and Ortega-S 2006). As with most deer management strategies, the success of habitat manipulation is dependent on local environmental conditions (weather, species diversity, composition of native flora, etc...) and the specific goals for the proposed plan. To effectively alter the landscape in favor of white-tailed deer, managers must assess the habitat requirements of local populations

and be aware of all possible ecological consequences that may result from this type of intervention (Fulbright and Ortega-S 2006).

c. Deer Management in Urban Settings

Urbanized landscapes provide food sources and protection from hunting (Cote *et al.* 2004), and deer populations in these areas can grow to reach remarkable densities. When human land-use overlaps deer habitat, conflicts can arise that can be harmful to humans and deer. Deer vehicle collisions for example, contribute to human and deer mortality and result in monetary losses in the billions for the US (Cote *et al.* 2004). Crop damage and overbrowsing cause conflicts when deer densities become too high (Waller and Alverson 1997) near agricultural lands, or in residential areas where deer can find nutritional food sources in gardens and lawns. Moreover, zoonotic diseases like Lyme disease and ehrlichiosis are more easily transmitted when deer densities are elevated (Cote *et al.* 2004), and such health concerns oftentimes contribute to negative human perceptions of deer in urbanized areas (Kilpatrick and LaBonte 2003).

For deer managers, these conflicts can be difficult to resolve because wildlife management in urbanized areas is complex and challenging (Roseberry and Woolf 1991). For one, management strategies used in rural settings, like hunting, are hazardous and oftentimes prohibited near cities. Additionally, landscape partitioning in developed neighborhoods can prevent management at relevant spatial scales (Brown *et al.* 2000), as many state agencies manage areas >1000 km^2 (Porter and Underwood 1999). Since habitats are more subdivided in urban compared to rural settings, more landowners will be affected by deer population control efforts. This presents a challenge because social acceptability of harvest can be lower in urban and suburban settings (Brown *et al.* 2000). Managers have to consider differences in deer acceptability that arise among stakeholders (Roseberry and Woolf 1991) since they undoubtedly

influence management decisions, and in some cases stakeholders have controlled the management outcome (Decker and Chase 1997). Ultimately managers must determine if the ecological and economic impacts of deer are sufficiently devastating to warrant population control in urbanized areas (Cote *et al.* 2004), a decision that involves values as well as science (Halls 1984).

One strategy for reducing overabundant herds in urbanized areas is to permit special hunts with limited entry and strict regulations. Some communities have resorted to selecting hunters based on skill, or temporarily permitting hunting in public parks while restricting harvest in close proximity to residential developments (Brown *et al.* 2000; Kilpatrick and LaBonte 2003). To circumvent firearm laws, managers have used bow hunting to reduce deer densities in suburban areas. Bow hunting proved to be effective at reducing deer densities in Fleming Park, near Kansas City, Missouri in 1994, and in Irondequoit, New York around the same time (Brown *et al.* 2000). Although, in Rock Cut State Park near Rockford, Illinois, bow hunting alone was not as successful at reducing deer to a desired density, and sharpshooters had to further reduce the herd after the bow season (Ver Steeg *et al.* 1995).

In the last several decades, the distribution and density of deer has continually increased and recently, it was realized that in some regions of the United States, hunting alone will not be able to reduce the deer population to tolerable levels (Brown *et al.* 2000). To further exacerbate this issue, hunter participation has declined in most states in the last decade (Enck *et al.* 2000), and so managers must seek alternatives for controlling overabundant deer in urbanized areas. Translocating deer to from urban to rural areas is one option for population control, though Beringer *et al.* (2002) found that this method was expensive and resulted in increased mortality for deer in Missouri (Beringer *et al.* 2002). Sharpshooting in urban areas has proven to be more

successful at reducing densities, but again, this method can be costly (DeNicola *et al.* 1997). Surgical sterilization can reduce deer reproduction in urban areas, but it is expensive (Mathews *et al.* 2005) and oftentimes controversial (Brown *et al.* 2000), thus further evaluations will be needed to determine the efficacy of such invasive measures.

Conclusions

As a society, we rely on deer managers to provide opportunities for hunting and wildlife recreation. When necessary, they are also expected to alleviate economic and ecological burdens caused by deer (Brown *et al.* 2000). Deer managers have to be educated in public policy as well as wildlife biology and ecology, because management goals have both social and biological components. Managers are also responsible for educating the public about the ecological consequences of management objectives, especially when the demands of society are not in the best interests of the herd (McCullough 1984; Porter and Underwood 1999). Expectations of deer managers are incessant, and as deer and human populations continue to grow, local state and federal agencies will face additional challenges requiring innovation and research to resolve. Though the public is not always aware of their value, wildlife managers are an asset. Deer management provides ecological, cultural, and economic benefits to society, and it is imperative for conserving deer as a natural resource (Langenau Jr. *et al.* 1984).

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Chapter 2: Utilizing CWD Surveillance to Examine Gene Flow and Dispersal in White-tailed Deer

Abstract

Prevention and management of transmissible diseases hinges upon understanding dispersal because it influences the distribution of wildlife species, determines interconnectivity among animals, humans, and pathogens, affects the rate of disease transmission, and alters the spatial distribution of infection. Understanding the relation between dispersal and chronic wasting diseases (CWD) was of interest because it can reveal patterns of disease spread and thus offer guidance for disease management. We used molecular techniques to examine dispersal in a population of Illinois white-tailed deer (*Odocoileus virginianus*) that has been intensely managed and hunted for decades. The majority of sampled individuals inhabited areas with confirmed cases of chronic wasting disease (CWD), a transmissible prion disease of cervids, with additional sampling in uninfected locations. We genotyped 1,410 deer harvested through CWD surveillance using 10 microsatellites and measured gene flow, determined population structure and quantified gender-specific differences in dispersal patterns. Additionally we used spatial autocorrelation and parentage assignments to examine movement of individuals at varying spatial scales. Female deer consistently demonstrated significant philopatry as evidenced by higher levels of genetic structure, positive spatial autocorrelation and maternity assignments within one home range. Male deer were less genetically structured and frequently exchanged genes across >100 km. Our results have shown that samples collected for disease surveillance can also be used to examine gene flow and dispersal in white-tailed deer. Our research indicates male dispersal and female philopatry across mixed agricultural and highly urbanized landscapes. Male deer have great potential to disperse over large distances (>100 km) in these complex landscapes, and such

long-distance movements could easily spread CWD to uninfected populations outside the endemic area in northern Illinois. Further, our results suggest that disease spread parallels gene flow, thus supporting the concept of horizontal transmission of CWD following dispersal of infected animals. These findings enhance our understanding of deer ecology and management and provide novel information about the potential for dispersal-induced spread of wildlife diseases.

Introduction

Dispersal has long been considered the foundation of ecology (Andrewartha and Birch 1954) and is equally important to epidemiology as it determines interconnectivity among animals, humans, and zoonotic pathogens. As wildlife hosts and vectors move through the landscape they influence rate of disease spread, spatial extent of infection and likelihood of new outbreaks (Cullingham *et. al* 2008). Because it impacts spatial and temporal disease dynamics, dispersal must be carefully considered when implementing disease control strategies as it will influence the effectiveness of management programs. Interconnections between disease and dispersal can make wildlife management difficult particularly when interspecies contacts are elevated in landscapes where urbanization, agriculture and wildlife habitats overlap (Bennett *et al.* 2006). Despite its importance from an ecological standpoint, dispersal remains one of the most difficult life history traits to quantify (Wiens 2001).

Understanding dispersal of white-tailed deer (*Odocoileus virginianus*) is critical for successful management of CWD, a prion disease of cervids. CWD in North America has caused economic losses associated with reduce hunting of an affected herd, depopulation of farmed cervids and losses of international markets among others (Arnot *et al*. 2009; Heberlein and Stedman 2009). Many outbreaks of CWD in the U.S. occur near cities and agricultural farms

which can create opportunities for zoonotic prion disease transmission (Macdonald and Laurenson 2006). While CWD associated prion disease has not been documented in humans, transmission of Bovine Spongiform Encephalopathy (BSE) as variant Creutzfeldt-Jakob disease (vCJD) and experimental infection of cattle with CWD suggest that species barriers for prion diseases are limited (Belay *et al.* 2004). CWD management is therefore essential not only to protect wildlife resources, but also to minimize risks to humans and livestock. As a result several states have implemented disease management plans to prevent CWD spread in captive and wild cervids. Wildlife managers need information on deer movement to determine risk for prion spread among wildlife habitats and into urban and agricultural landscapes to implement the most effective strategies for disease control.

Until recently, deer managers relied exclusively on radiotelemetry to provide direct estimates of dispersal. In general, these studies suggested that dispersal is male-biased with fawns and females demonstrating philopatry (Marchinton and Hirth 1984). Dispersal distances for radio-collared deer in Illinois ranged from 28-44 km and most deer did not disperse >50 km (Nixon *et al.* 2007). Unfortunately, telemetry studies in deer are labor intense. Moreover, they are limited by sample size, study area, and the difficulties of trapping a random sample (Koenig *et al.* 1996). Recently, genetic measures of dispersal have gained popularity because population-level movements of targeted species can be examined across entire landscapes to provide essential information for ecosystem-based management plans. By studying distributions of alleles within and among populations, gene flow can be measured and genetic structure examined so that habitat connectivity can be assessed (Berry *et al.* 2004). Furthermore, population-based techniques have been enhanced by individual-based Bayesian assignment tests

(Pritchard *et al.* 2000) and parentage analyses (Kalinowski *et al.* 2007) that assign individuals to demes, and quantify geographic distances between parent-offspring pairs.

We applied genetic methods to indirectly elucidate movements of white-tailed deer, a species that has been intensely managed and hunted for decades (Pietsch 1954; Calhoun and Loomis 1974). In this study we utilized tissues from deer harvested through CWD surveillance and management programs aimed at reducing further CWD transmission. Using these samples our goals were to:

1. Examine population-level movement and test for male-biased dispersal by quantifying differences in allele frequencies among populations.
2. Identify genetic clusters and determine admixture with individual-based Bayesian assignment tests.
3. Examine dispersal using spatial autocorrelation and parentage assignment.
4. Evaluate the feasibility of indirectly measuring dispersal using samples collected through wildlife management programs.

Our study provides a rare opportunity to assess indirect measures of deer movement in the context of wildlife disease management. Our genetic evaluation of behavior also allows comparison to previous studies of deer dispersal employing direct measures, thus broadening and extending our understanding of deer ecology and contributing to adaptive management of wildlife disease in hunted-harvested species.

Materials and Methods

Deer Sampling

We utilized tissue samples collected through CWD surveillance and population control programs targeting four areas in Illinois: north-western, north-central, north-eastern, and east-central. The

majority of samples were collected from areas at increased risk for disease based on proximity to prior CWD cases to improve confidence in disease detection and evaluation of management strategies (Thurmond 2003). Additional samples were from areas of special management interest. Harvest date, gender, age and spatial locations were collected for all samples.

North-central Illinois sampling area (NIL). Between January 2003 and March 2008, Illinois Department of Natural Resources (IDNR) harvested ~5,000 free-ranging deer in the CWD-infected region of northern Illinois (Fig. 2.1 and inset). Of the samples collected, 814 from Winnebago, Boone, DeKalb, Ogle, and McHenry counties were used for genetic analysis. For deer sampled in NIL, spatial locations were recorded to the nearest township/ range/section (TRS). For population-level analyses the NIL sampling area was subdivided into 13 similarly-populated study sites based on geographic distribution of sampled animals (Fig 1. and inset).

North-western Illinois sampling area (GTA). Between January and February 2008, 222 free-ranging deer were harvested in population control programs from Galena Territory Association in JoDavies County. For deer sampled in GTA, spatial locations were recorded to the nearest 0.1 km.

North-eastern Illinois sampling area (DuP). Between December 2007 and January 2008, 50 free-ranging deer were harvested by United States Department of Agriculture Wildlife Services personnel through deer management programs initiated by the DuPage County Forest Preserve. For deer sampled in DuP, spatial locations were recorded to the nearest township/ range/section (TRS).

East-central Illinois sampling area (RAP). During fall hunting seasons between 2005 and 2007, 324 free-ranging deer were harvested at University of Illinois Robert Allerton Park (RAP)

through their Deer Management and Research Program. For deer sampled in RAP, spatial locations were recorded to the nearest km.

Laboratory Procedures

Muscle samples were stored in 100% ethanol and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison WI). Individuals were genotyped using microsatellite DNA primers previously developed for white-tailed deer (Anderson *et al.* 2002; Blanchong 2003). This panel included markers BM 1225, BM4107, CSN3, (Bishop *et al.* 1994), IGF-1 (Kirkpatrick 1992), OBCAM (Fries *et al.* 1993), OarFcb304 (Buchanan *et al.* 1993), RT 20, RT 23, RT 27 (Wilson *et al.* 1997) and Srcrsp-10 (Bhebhe *et al.* 1994). Mutations in microsatellite flanking regions required the re-design of primers CSN3 (reverse primer = TAGCTCATAATGTAAACCACTTT) and RT 20 (forward primer = TGGAAGATTTCAGAAATGAT). Forward primers were labeled with fluorescent dyes (NED, HEX, FAM) with fragments separated on an ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA) and visualized with GeneMapper (v. 4.0: Applied Biosystems, Foster City, CA). MicroChecker (v.2.2.3; Van Oosterhout *et al.* 2004) was used to evaluate genotyping errors using expected allele frequencies derived under Hardy-Weinberg Equilibrium (HWE).

Descriptive Statistics

Expected heterozygosity was calculated for each locus in each of the four sampling areas (with all 13 NIL study sites pooled) using Arlequin version 3.1 (Excoffier *et al.* 2005). Allelic diversity and the number of rare alleles per locus (alleles exclusive to one sampling area) were determined with deviations from HWE examined using F_{IS} (inbreeding coefficient) by locus and overall with GenePop (v.4.0; Rousset 2008).

Population-Level Movements

We computed F_{ST} values among study sites with Arlequin (Excoffier *et al.* 2005) and used Mantel tests to examine correlations with geographic distance (in km) using Isolation By Distance Web Service (IBDWS v.3.16; Jensen *et al.* 2005), with significance based on 1000 random permutations. Isolation-by-distance (IBD) was examined at two spatial scales: distances <100 km including all thirteen NIL study sites and distances <300 km encompassing NIL, DuP, GTA, and RAP study sites. When performing tests for IBD, we analyzed both genders combined, then each separately.

We tested for sex-biased dispersal using FSTAT (v.2.9.3.2; Goudet, 2001) with F_{ST} values calculated separately for males and females at <100 km and <300 km spatial scales and significance determined by 1000 random data-permutations. To further define gender-specific population boundaries, our 16 study sites (Fig. 2.1) were coalesced into populations based on homogeneity of allele frequencies determined by TFGPA (v.1.3; Miller 1997). To approximate probabilities associated with observed allele frequencies for each locus, χ^2 tests were calculated from contingency tables of allele frequencies among all pairwise combinations of study sites and significance evaluated using Markov Chain Monte Carlo (MCMC). For each single-locus test, 5000 permutations were completed for each of 20 batches with 1000 dememorization steps. *P*-values from single-locus tests were pooled using Fisher's Combined Probability Test and multi-locus *P*-values (<0.05) were evaluated for departures from homogeneity in allele frequencies between populations. To prevent multi-locus tests from being dominated by a single marker, single-locus *P*-values were minimized at $P = 0.0001$ (Waples and Gaggiotti 2006).

Study sites not significantly different from one another in the contingency tests were then combined as samples representing the same genetic population. Thus, populations may consist of

one to several study sites for which all other multi-locus tests were significant, or instead as multiple study sites linked through a series of non-significant tests (Waples and Gaggiotti 2006).

Bayesian Assignment of Individuals

We also inferred population structure using an individual-based Bayesian clustering method implemented in STRUCTURE (v. 2.3.1; Pritchard *et al.*, 2000) that estimates the natural logarithm of the probability [LnP(D)] that individual genotypes belong to a given cluster (*k*), thus negating the need for *a priori* population definitions. Twenty replicates were run for *k* values 1 through 7 and the simulation yielding the smallest Bayesian deviance was selected as the optimal cluster model (Pritchard *et al.* 2000; Faubet *et al.* 2007). An admixture model (initial α =1.0, max α = 10.0, SD of α = 0.05) was computed with TRS as the location for each deer and correlated allele frequencies specified to account for shared ancestry (Pritchard *et al.* 2000; Falush *et al.* 2003). Initially, several pilot simulations were run for each dataset to determine burn-in length and consistency of parameter estimates across replicates. Male and female clusters were then simulated separately using a burn in of 100,000 MCMC steps followed by 100,000 replicates to estimate posterior probabilities of all parameters. Individuals were assigned to the inferred cluster containing the highest percentage of membership (*q*).

Since males were not genetically structured at spatial scales <100 km, male deer sampled from all 16 study sites were included in the analysis. Females on the other hand, were genetically heterogeneous at distances <100 km, so geographic subsampling was used to confirm results at varying spatial scales (Pritchard *et al.* 2000; Falush *et al.* 2003). Results from subsampled data sets were qualitatively similar to models including all females, and thus are not reported.

Spatial Autocorrelation

Tests for global spatial autocorrelation were performed with individuals from NIL and DuP using the single population procedure in GenAlEx (ver. 6.2; Peakall and Smouse 2006). Those from GTA and RAP were omitted due to inadequate sampling at intermediate distances. Individuals were grouped by gender and age class (fawn, yearling or adult) so that sex-biased dispersal and juvenile movements could be examined. For all individuals, pairwise geographic distances were calculated from the x/ y-coordinates of the TRS centroid (2.6 km^2) from which they were sampled. Pairwise geographic and squared genetic distance matrices were used to calculate *r*, the autocorrelation coefficient for each group at distances ranging from 0.5-200 km. A one-tailed distribution was derived from 999 random permutations and bootstraped to determine significance and 95% confidence limits. Distance classes were 0-0.5 km (within a TRS), 0-2 km (between adjacent TRS), 0-3 km (separated by 1 TRS), 0-6 km, 0-12 km, 0-24 km (~ 1 dispersal event; Nixon *et al.* 2007), 0-48 km, 0-100 km (long-distance dispersal). Sample sizes for each group at each of the 9 distance classes are in Table 2.2.

Parentage Analysis

Mother-offspring pairs were identified using Cervus 3.0 (Kalinowski *et al.* 2007). Cervus employs a multi-step process to first simulate the distribution of log-likelihood values for mother-offspring pairs using allele frequency data from sampled populations, and second determine statistical confidence of parentage for sampled individuals. To ensure conservative parentage estimates, an error rate of 0.001 was used (observed error rate= 0.005, data not shown) in simulations of 100,000 offspring. All females were used as candidate mothers while all individuals were considered potential offspring with subsequent removal of duplicate parentage assignments for a single pair of individuals. We validated the ability of our markers to resolve

parentage by including 100 confirmed mother-offspring pairs (mothers with fetuses in utero). Based on simulations of the observed data and validation with mother-offspring pairs assignments at 90% confidence were evaluated. Spatial distances among mother-offspring pairs were determined using the geographic distance calculation in GenAlEx.

Results

Descriptive Measures

Multi-locus genotypes were obtained for 1,410 deer across 4 sampling areas in the northern half of Illinois. None of the 10 microsatellites violated HWE assumptions, suggesting that null alleles and large-allele drop-out did not affect scoring. Only one marker in NIL showed significant heterozygote deficiency, but Fis was low (Fis = 0.019) suggesting subpopulation structure rather than inbreeding. Global tests showed no evidence of heterozygote excess in any of the sampling areas. Number of alleles per locus (average = 12) ranged from 2 (OarFcb304) to 19 (RT23), with 15 rare alleles observed across 6 loci in NIL and RAP (Table 2.1).

Population-Level Movements

IBD was detected for all individuals at 100 km (Mantel r =0.39, *P*=0.03), but not at 300 km (Mantel r =0.27, *P*=0.11). When genders were analyzed separately, females demonstrated significant IBD at the 100 km spatial scale (Mantel r =0.37, *P*=0.04), but not at 300 km (Mantel r =0.15, *P*=0.24). Males did not exhibit IBD at either spatial scale (Mantel r =-0.03, and 0.20, *P*=0.54 and 0.15, respectively).

Average female F_{ST} at 100 km (F_{ST}=0.0122) was significantly larger than that of males (F_{ST}=0.0054; *P*=0.004), underscoring male-biased dispersal (per Marchinton and Hirth, 1984). Similar patterns were observed at 300 km, with females genetically more structured than males, again with a significantly larger F_{ST} (*P*=0.009; Fig. 2.2).

Males revealed weak genetic structure based on contingency tests for heterogeneity of allele frequencies among study sites. Two (of 16; 12.5%) study sites (RAP and GTA) were significantly distinct populations (P<0.05). NIL and DuP study areas were linked through a series of non-significant tests, thus indicating a single panmictic population (Fig. 2.3).

Females were significantly more structured than males across the 16 study sites, with 7 genetically heterogeneous populations (43.75%; Fig. 2.3). Allele frequencies in RAP, GTA, and four NIL study sites were heterogeneous (P<0.05) when compared to all other study sites. The remaining 9 NIL and DuP study sites were compiled into one genetic population through a series of non-significant tests.

Bayesian Assignment of Individuals

Consistent with results from contingency tests, for males a 3-cluster model produced the lowest deviance value in STRUCTURE (Fig. 2.4). The first cluster included RAP, a second GTA, and a third DuP + all 13 NIL study sites (inferred clusters denoted by italics and subscript "$_i$"). Figure 3 shows the spatial distribution of males assigned to each of the three clusters, with misassignment rates ranging from 0 (RAP, GTA) to 31% (NIL study site 7) (Fig. 2.3). Males from RAP were the most genetically distinct (90% membership therein), whereas GTA was admixed with 66% assigned therein, 18% to RAP_i and 16% to the NIL/DuP_i. Males from NIL and DuP had intermediate levels of admixture with 71% to 51% membership assigned to NIL/DuP_i. Overall, southern study sites were more admixtured than northern sites in NIL/DuP_i. Study sites assigned to NIL/DuP_i were of mixed ancestry with RAP_i, in that 12 (of 14) study sites reflected RAP_i rather than GTA_i membership. Across all replicates at k=3 clusters, male assignments were consistent, membership estimates stable, and all inferred clusters were in HWE for all loci (P<0.01).

For females, 4 clusters produced the lowest Bayesian deviance value (Fig. 2.4). Although all 4 were in HWE ($P<0.01$), study site assignments varied within replicates, and membership proportions were inconsistent. Consistent with population boundaries determined through contingency tests, the best fit model placed RAP into one cluster, GTA into a second cluster, and DuP + 9 NIL study sites north and east of Rockford into a third cluster. The last inferred cluster ($NILSW_i$) however, contravened contingency tests by amalgamating four genetically heterogeneous populations to the south and west of Rockford into one cluster. Figure 3 shows the spatial distribution of individuals assigned to the 4 inferred clusters. Misassignment rates for females ranged from 0% to 47% (NIL study site 5). Females from the RAP study area were the most genetically distinct (at 91% membership), whereas females from GTA were also highly differentiated (at 86% membership) with the remaining 14% assigned preferentially to the NIL/DuP_i cluster. For study sites in NIL/DuP_i and $NILSW_i$ clusters, admixture became elevated as study sites approached Rockford, a pattern consistent with IBD and clinal admixture in this area.

Spatial Autocorrelation

According to 95% confidence limits determined by bootstrapping, adult males and male yearlings were not spatially structured at distances up to 100 km, though yearlings were structured at 0-6 km (Fig. 2.5). At ≤0.5 km, male fawns demonstrated the strongest positive spatial autocorrelation of any group examined, averaging $r=0.041$for this distance class. For male fawns, significant autocorrelation was maintained at distances ≤3 km, then declined sharply. Adult females also showed positive spatial autocorrelation that was maintained at distances ≤48 km, with gradual decreases in r observed with increasing distance (Fig. 2.5). Female yearlings were not spatially autocorrelated at any distance, and average r values were

similar to those observed in adult males. Like male fawns, female fawns were also significantly autocorrelated, though female fawns showed significant structure at larger distances (≤24 km).

Parentage analysis

Only 4% of non-mothers were assigned maternity at 90% confidence when the true mother was not sampled, and 6% when the true mother was sampled. All mother-fetus pairs used for validating parentage were correctly assigned at 90% confidence levels. For individuals with unknown parentage, Cervus assigned 10 mother-offspring pairs at 90% with no mismatches across all multilocus genotypes. Of the 10 pairs assigned 5 were mother-daughter pairs and 5 were mother-son pairs. All assigned male offspring were ≤ 2 years old, with 80% of mother-son pairs being fawns. Two mother-daughter pairs included fawns, while the remaining 3 assignments were between adult females. Distances between assigned pairs ranged from 1 to 4 km with an average distance of 2.9 km. Half of the mother-offspring pairs detected were within the same home range, while the other half were separated by distances equivalent to adjacent home ranges (as estimated by Nixon *et al.* 1991 for deer in RAP).

Discussion

Predicting the potential spread of wildlife diseases and identifying areas at high risk for infection hinges upon quantification of animal dispersal (Castillo-Chavez and Yakubu 2001). Many ecological and epidemiological studies have focused on wild cervids because the elevated mobility of this species can rapidly spread disease to new locations (Blanchong *et al.* 2007; Cross *et al.* 2007; Conner *et al.* 2008). Furthermore, cervids frequently cohabitate in pastures with livestock and also reside in urban areas, thus providing opportunities for zoonotic disease transmission (Dazak *et al.* 2000). Utilizing genetic samples obtained through disease surveillance, we quantified deer movements in areas infected with CWD. Admixture proportions

calculated from assignment tests suggest that long-distance dispersal (≤300 km) occurs and that CWD could easily spread to GTA and RAP through occasional long-distance movements. These findings implicate long-distance dispersers as potential carriers of disease, and are consistent with the current distribution of CWD in Illinois where single isolated cases are detected >100 km from the outbreak focus near Rockford (Fig. 2.1; IDNR 2008).

Our genetic analyses suggest that males have great potential to perpetuate disease in infected areas because of their extensive local dispersal (<100 km). More importantly, the genetic structure of males suggest that a substantial proportion disperse >100 km, which in turn, implicates them as vehicles for long-distance transmission. In contrast, spatial autocorrelation and parentage assignments revealed that generally females are philopatric, indicating that they are more likely to spread infectious diseases within their cohort. Yearling females however, were inclined to disperse as autocorrelation was not apparent in this age class. This suggests that dispersing females leave natal ranges one year after birth, and would therefore be more likely to spread disease than fawns or philopatric adult females. Further, our findings identify areas at highest risk for pathogen influx via immigration. Admixture between deer from northern Illinois and RAP deer was higher than admixture between northern Illinois and GTA, suggesting that genetic exchange is elevated to the south rather than west, a pattern consistent with the tendency for southerly spread of CWD. Though preliminary, disease spread appears to parallel gene flow, and our results support the concept of horizontal transmission of CWD following dispersal of infected animals.

Our data consistently showed that genetic structure in deer is shaped by profound differences in gender-specific dispersal with male-biased dispersal evident in F_{ST} values and spatial autocorrelation patterns. Overall males were genetically homogeneous locally (<100 km)

and substantially admixed regionally (<300 km), which in turn demonstrates their enhanced dispersal capabilities. Females, on the other hand, were genetically structured locally (<48 km) according to spatial autocorrelation and contingency tests, indicating reduced connectivity among philopatric subpopulations. However, in conjunction with Bayesian assignment tests and IBD, these results suggest that females in NIL behave as a cline and not as independent subpopulations. While females on opposite sides of NIL were genetically heterogeneous, substantial genetic admixture was detected along contact zones between genetically heterogeneous groups of females on opposite sides of NIL. Collectively, female philopatry is responsible for genetic structuring at distances <100 km whereas male dispersal is primarily responsible for connectivity among habitats separated by ≤300 km.

Our results also indicate that males and females respond differently to habitat fragmentation induced by urbanization, suggesting the spread of disease into urbanized areas may be gender-based. We detected barriers for dispersal in females between the most geographically proximate study sites using F_{ST}, assignment tests and contingency tests. Female structure corresponded to habitat isolation induced by an interstate freeway (I-39: a 4 lane, divided and fence roadway with limited vehicle access) built in the 1980s. However, males reflected homogeneity of allele frequencies at these same study sites (Fig. 2.3) suggesting the freeway does not limit connectivity for males at this location. Furthermore, the panmictic population quantified in northern Illinois implies that males disperse freely within this 6900 km^2 area, apparently undeterred by the presence of Rockford, the third largest city in Illinois. Females, on the other hand, were inhibited by Rockford, as female gene flow was reduced and separate populations were detected on each side of this urbanized area. Males, given their

insensitivity barriers, would be more likely to move through urbanized areas, thus elevating the risk for zoonotic disease transmission.

Our results for females apparently contradict those from a deer study in Wisconsin (Blanchong *et al.* 2007) where female dispersal was not deterred by roads. However, the road was a highway (US18/151: a divided 4-lane road, but with high vehicle access) which presents less of a physical barrier than an interstate freeway. Our results are consistent with Epps *et al.* (2005) who showed that gene flow in big horn sheep (*Ovis canadensis*) was truncated by interstate highways while Pérez-Espona *et al.* (2008) found that red deer movement was deterred by high traffic roads. These anthropogenic alterations of both deer and sheep habitats promoted genetic structuring over a period of 20-40 years (Epps *et al.* 2005).

Dispersal, as quantified in our study through gene flow, did not always agree with direct estimates of dispersal for Illinois deer. STRUCTURE results showed that ongoing genetic exchange occurs at distances > 200 km (Fig. 2.3). Though long-distance dispersals (~200 km) are occasionally documented in ecological studies of deer (Kernohan *et al.* 1994; Brinkman *et al.* 2005; Oyer *et al.* 2007), those >250 km exceed distances recorded in any North American ecological study to date. Further, while male-biased dispersal was detected with our genetic data, telemetry data suggests that both sexes disperse in Illinois (Nixon et al 1991; Hansen *et al.* 1997; Nixon *et al.* 2007). In agricultural habitats of the midwestern North America similar proportions of radio-collared deer dispersed from their natal ranges in RAP (57% of males and 49% of females), and in DeKalb County (68% of males and 45% of females) (Nixon *et al.* 2007). Goudet *et al.* (2002) argued that sex-biased dispersal must be stronger than that levels documented (for example) by Nixon *et al.* (2007) before it can be detected using genetic data. However, our study was performed at the population-level and included >1400 deer sampled across the northern half

of Illinois. Given this, we were able to detect long-distance dispersals and subpopulation processes that are often overlooked in short-term ecological investigations.

Historic averages of dispersal are incorporated into estimates of gene flow when molecular markers, like microsatellites, are used (Bohonak 1999). Management agencies in the early twentieth century restocked herds following a near extinction by translocating deer from remnant populations in north-central and southern Illinois to eighteen counties across the state (Pietsch 1954; Calhoun and Loomis 1974). These translocation distances are uncharacteristic of normal deer dispersal (Nixon *et al.* 2007) and may have influence contemporary genetic structure. Deer in Mississippi were also re-populated in the early 1900's following an anthropogenically-induced population crash, and resultant effects on genetic structure are comparable to those in our study (DeYoung *et al.* 2003). Similarly, re-introductions and serial translocations of red deer (*Cervus elaphus*) in Scotland and England contributed to genetic homogeneity of populations across ~350 km of habitat (Hmwe *et al.* 2006).

We recognize that discretion must be used when gene flow estimates are employed as a surrogate for dispersal in highly mobile organisms, especially with samples obtained from disease surveillance. In Illinois, surveillance is concentrated in areas known to harbor CWD or areas experiencing overabundance. Therefore, uneven sampling across the study area may have affected the detection of genetic structure at local scales. Because we sampled to detect disease, rather than sampling continuously across the landscape, the likelihood of detecting mother-offspring pairs and spatial autocorrelation was not equally probable in all directions. Hence, while parentage analysis allowed us to detect mother-offspring pairs within a single home range, our sampling distribution most likely prevented detection of mother-offspring pairs at larger distances. Nonetheless, we applied conservative criteria to detect parentage and spatial

autocorrelation in that simulated error rates for parentage were 5 times lower than observed, and conservative bootstrap confidence limits were used to evaluate autocorrelation. In addition, when performing spatial autocorrelation and parentage, we carefully subsampled the data and omitted deer from GTA and RAP so as to prevent bias due to clustered sampling.

The practice of culling CWD-positive herds to minimize risk of spreading the disease has raised some concerns about negative impacts on population viability. However, we did not detect population bottlenecks, and allelic diversity was equal to (or greater than) that documented in other regional studies (Anderson *et al.* 2002; Blanchong *et al.* 2003; Doerner *et al.* 2005), suggesting that reductions in deer abundance by culling did not result in an observable loss of genetic diversity within the time frame examined. More importantly, as predicted by theoretical studies (Lloyd-Smith *et al.* 2005) and modeling scenarios (Wasserberg *et al.* 2009) to date, culling as a management strategy has lowered prevalence in CWD-positive areas in Illinois and minimized its spread to other areas (Weng, unpublished data). Both are a silver lining in an otherwise rather bleak prognosis on containment and potential eradication of CWD.

Our findings have shown that in heterogeneous landscapes such as Illinois, white-tailed deer populations are maintained by long-distance male dispersal and female philopatry. The profound differences in males and female genetic structure underscore the importance of utilizing several statistical approaches to quantify gene flow at varying spatial scales. With this comparative approach, we were able to make meaningful conclusions about the genders despite vast differences in their movement behaviors. This work contributes to an overall understanding of deer population genetics and wildlife disease while providing a larger context for the comparison of demography, behavior and genetic tendencies of deer in Midwestern North America. Furthermore, our study has shown that with careful analysis indirect estimates of

movement can be obtained from samples collected through disease surveillance, a finding that offers great potential for future studies examining the interplay between disease and dispersal. These data will benefit deer managers during implementation of hunting regulations, wildlife disease control, and the successful management of deer in urbanized areas.

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Table 2.1. Expected heterozygosity, sample size (*n*) and the number of alleles (both common and rare to one sampling area) detected in Illinois deer.

Study Site	*n* Females	*n* Males	Expected Heterozygosity*	Number of Alleles*	Number of Rare Alleles
NIL	485	320	0.72	117	8
GTA	150	60	0.7	99	0
RAP	226	76	0.72	113	6
DuP	19	17	0.73	87	0

*Averaged across 10 loci.

Table 2.2. Sample sizes (*n*) and number of pairwise comparisons at each distance class for global spatial autocorrelation of deer grouped by gender and maturity (M=male, F=female) in northern Illinois (NIL) and DuPage County (DuP).

		Distance Class							
Group	***n***	**0-0.5**	**0-2**	**0-3**	**0-6**	**0-12**	**0-24**	**0-48**	**0-100**
Adult M	90	96	135	146	286	582	1271	2402	3757
Yearling M	85	125	227	297	449	597	1179	2027	3342
Fawn M	164	340	590	772	1845	2650	5593	10226	13253
Adult F	299	1063	1843	2267	4671	7420	15152	29495	42508
Yearling F	69	126	174	189	270	402	726	1260	2228
Fawn F	179	753	1067	1259	2264	3029	5003	8495	11475

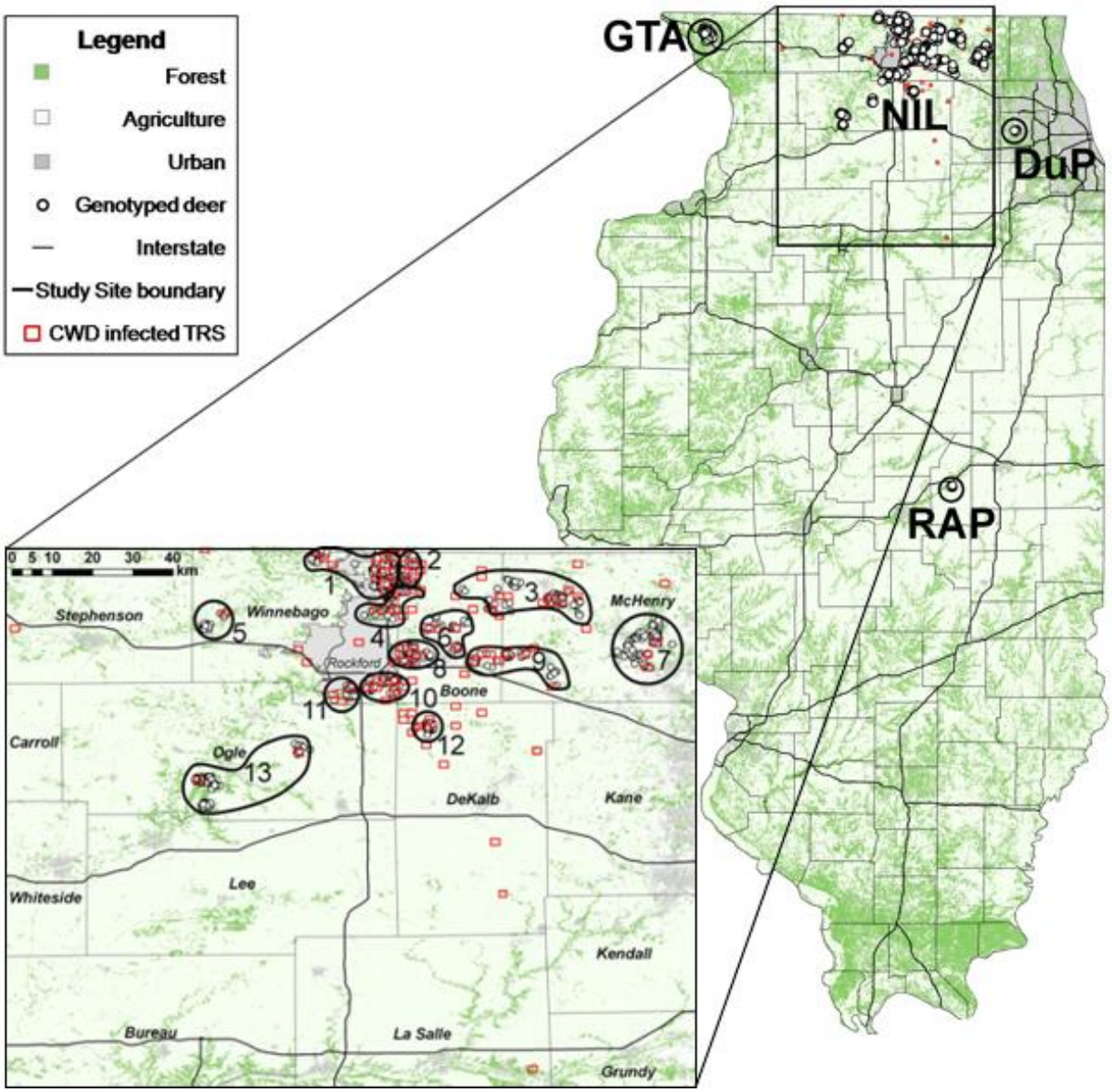

Figure 2.1. Illinois study areas. NIL=northern Illinois, GTA=Galena Territories Association, DuP=DuPage Country Forest Preserve, RAP=Robert Allerton Park. Inset map displays subdivision of NIL study area into 13 study sites, where each open circle represents one genotyped deer and each open red boxes indicates a CWD infected TRS.

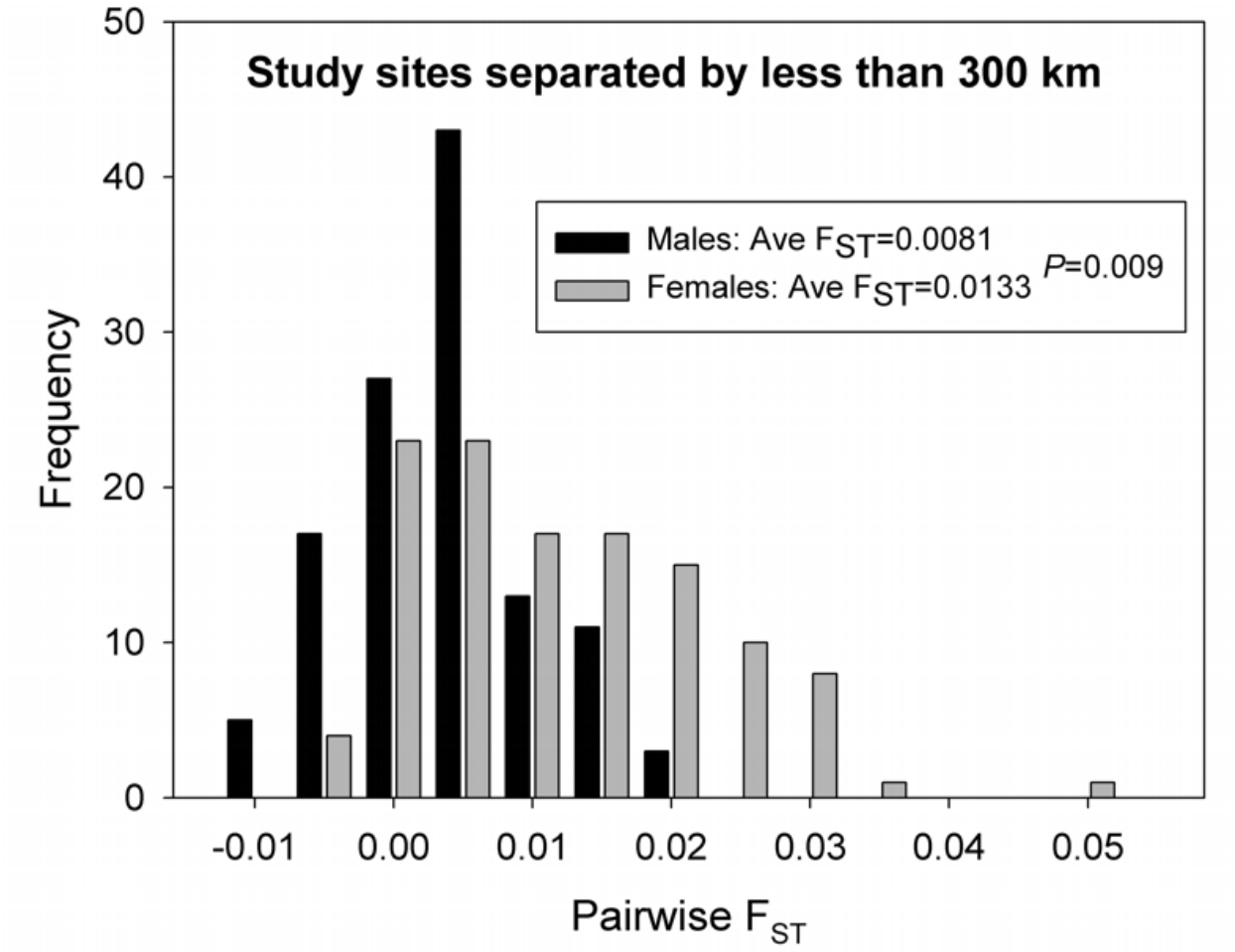

Figure 2.2. Frequency of pairwise F_{ST} values at 300 km. To test for sex-biased dispersal, average F_{ST} values were calculated for males and females and significance evaluated with 1000 random permutations of the observed data. Results from sex-biased dispersal tests are shown in the upper right.

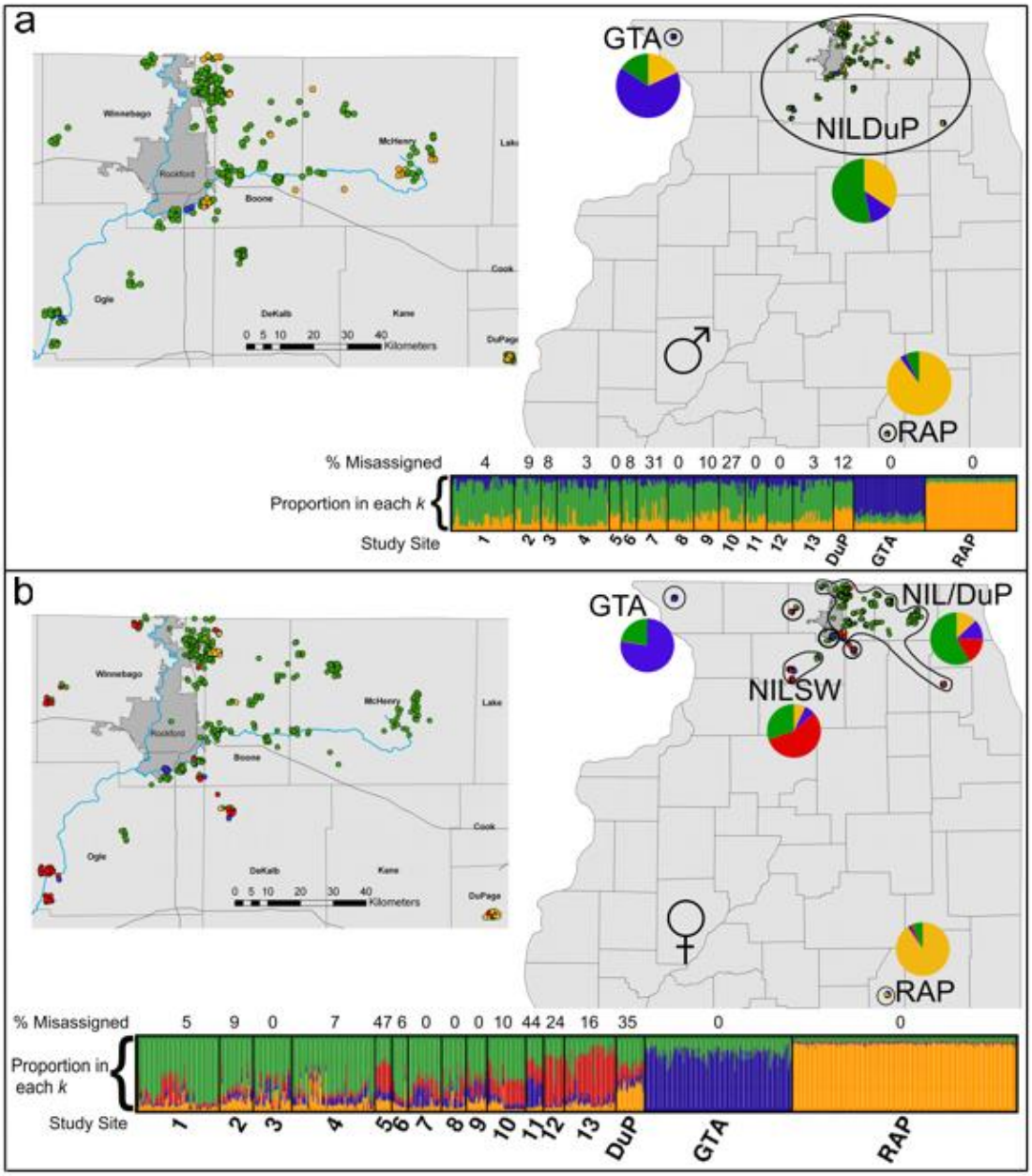

Figure 2.3. Genetic populations of male (a) and female (b) deer in Illinois as revealed by χ^2 tests for heterogeneity of allele frequencies (black outlines) and Bayesian assignment of individuals (colored dots in maps). Clusters were simulated using STRUCTURE and individuals were assigned to the inferred cluster which contained the highest percentage of membership. Membership proportions in each inferred cluster are shown for individuals (vertical lines for each individual; separated into study sites by black vertical lines) and also for inferred clusters (pie charts). Blue dots represent individuals assigned *GTA* membership, green dots represent individuals assigned *NIL/DuP* membership, red dots represent individuals assigned *NILSW* membership and yellow dots represent individuals assigned *RAP* membership.

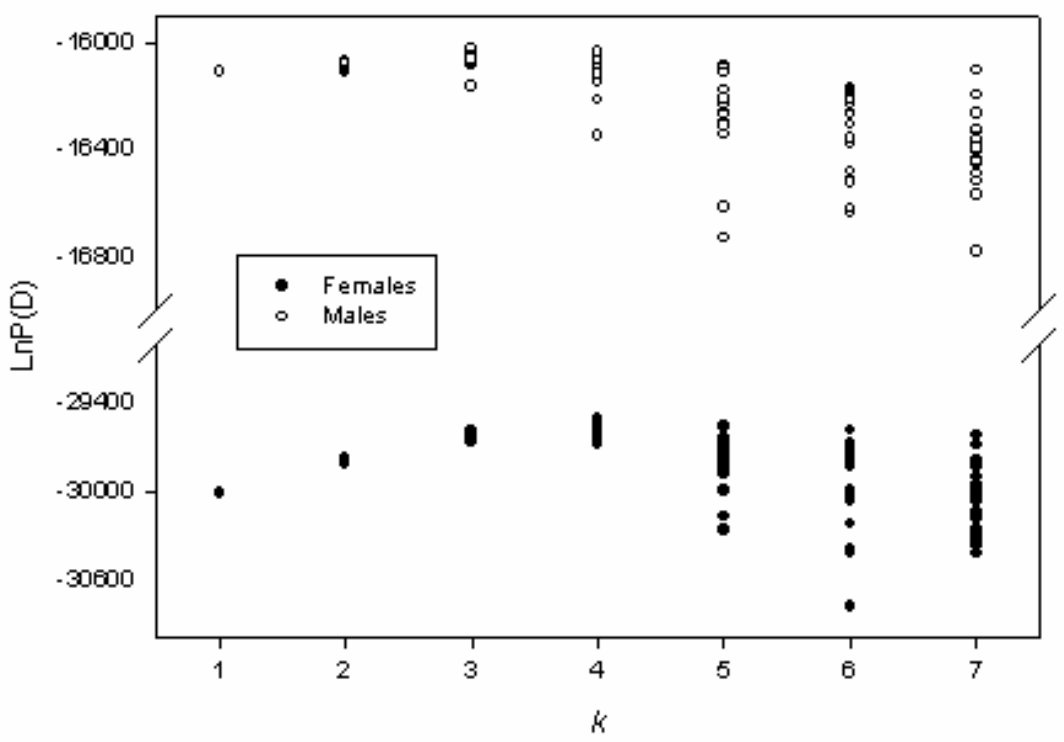

Figure 2.4. Distribution of likelihood values generated from 20 STRUCTURE replicates at *k* 1-7 for male and female deer in Illinois. LnP(D) is the natural logarithm of the probability (LnP(D)) that individual genotypes belong to a given cluster (*k*). All simulations were run with a burn in of 100,000 Markov chain Monte Carlo steps followed by 100,000 replicates to estimate posterior probabilities of all parameters. The model yielding the lowest Bayesian deviance value was *k*=3 for males and *k*=4 for females.

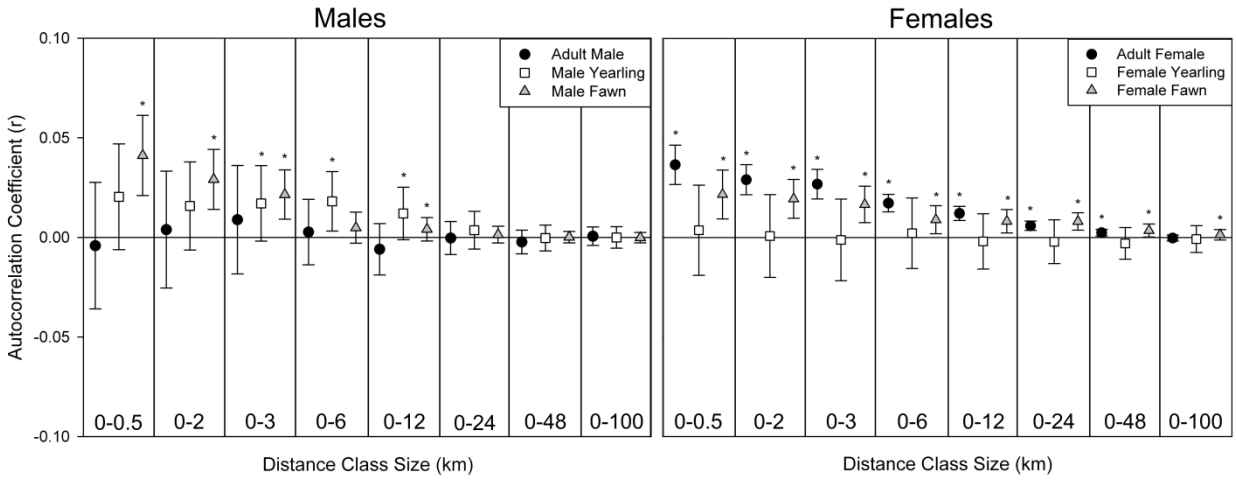

Figure 2.5. Global spatial autocorrelation for male and female deer grouped as fawn, yearling, and adult. Error bars surrounding the average *r* represent 95% confidence limits calculated with 999 bootstraps. * indicates significant ($P<0.05$) positive spatial autocorrelation determined through standard permutation tests.

Chapter 3: Null Alleles Behaving Badly: Empirical Evaluation of Genotyping Errors and their Impacts on Population Studies

Abstract

Genotyping errors can bias results, promote biological misinterpretations, and create severe consequences for studies that guide population management and conservation. We used empirical data from a large-scale microsatellite DNA study of white-tailed deer to identify sources of genotyping errors, evaluate measures to correct for their presence and provide recommendations to prevent their negative impacts. We detected null alleles in five of 13 previously evaluated microsatellites, and redesigned primers for two of these loci. Analytical corrections for null alleles were unable to fully prevent bias associated with these genotyping errors, and consequently, measures of population differentiation and kinship were negatively impacted. Our results demonstrate the importance of error evaluation during all stages of population studies, and emphasize the need to standardize procedures for genetic marker evaluation. Our findings are broadly applicable in that solutions are presented to prevent genotyping error and bias in all types of microsatellite-based population studies.

Introduction

Microsatellites have become increasingly popular as genetic tools in studies of ecology, kinship and evolution (Chapuis and Estoup 2007). They are extremely polymorphic and versatile, largely because of their exceedingly high mutation rate (10^3 times higher than functional genes) (Moxon and Wills 1999). Codominant inheritance and selective neutrality make microsatellites ideal for measuring genetic exchange among interbreeding groups or individuals, and when applied appropriately, they provide precise and statistically powerful means of quantifying genetic relationships (Dewoody *et al.* 2006). Hundreds of studies have now

applied microsatellites to examine ecological parameters like parentage (McLean *et al*. 2008), gene flow (Epps *et al.* 2005, Spear *et al.* 2005), effective population size (Wang 2009), and genetic relatedness (Lynch and Ritland 1999, Lunn *et al.* 2000). Moreover, recent innovations have greatly reduced labor and costs for microsatellite analysis (Lepais *et al.* 2006), making their application cost-effective even for studies using large empirical data sets.

While microsatellites are clearly beneficial and their applicability widespread, few studies fully recognize the technical shortcomings that can arise when they are employed to measure genetic exchange (Dakin and Avise 2004). Errors occur when the observed genotype is not equivalent to the true genotype (Pompanon *et al.* 2005). These most often result from the inclusion of null alleles that fail to amplify in PCR assays because of point mutations within primer annealing sites (Callen *et al.* 1993). Such variation in primer binding regions can result in invisible alleles that appear to defy Mendelian inheritance patterns (Callen *et al*. 1993). Null alleles can also result from technical problems associated with PCR, in that smaller alleles can be preferentially detected over larger alleles because of the efficiency of amplifying shorter PCR fragments. This inconsistency is often referred to as 'allelic dropout' or a 'partial null' and it is oftentimes associated with low DNA template quality (Pompanon *et al*. 2005)

Unrecognized null alleles, regardless of their origin, can have adverse consequences in ecological studies. Gagneux *et al.* (1997a) examined chimpanzee paternity with microsatellites and found that most females participated in extra-group copulations based on paternal exclusion of males within their social group (Gagneux *et al.* 1997b). However, this *Nature* article was retracted when the actual source of father-offspring mismatches was found to be null alleles (Gagneux *et al.* 2001). Gagneux *et al.* (1997b) used non-invasive techniques for sampling and cited low quality DNA as the cause for allelic dropout, which in turn yielded genotyping errors

(Gagneux *et al.* 1997a). This example demonstrates the broad and pervasive potential for biological misinterpretations that can result from genotyping errors, and underscores the importance of obtaining accurate baseline molecular information.

A substantial frequency of null alleles is manifested in a population as homozygote excess (heterozygote deficiency) that occurs when heterozygous null alleles fail to amplify and genotypes thus appear monomorphic (Pemberton *et al.* 1995). Usually homozygote excess can be detected through deviations from Hardy-Weinburg equilibrium (HWE), but in some instances it only becomes apparent when confirmed parent-offspring dyads seemingly have different homozygous genotypes resulting from shared heterozygous null alleles (Dakin and Avise 2004). Several software programs are designed to aid in the detection of genotyping errors by testing specifically for the presence of null alleles (i.e., MicroChecker: Van Oosterhout *et al.* 2004, GenAlEx: Peakall and Smouse 2006) or by identifying heterozygote deficiencies via HWE analysis (Microsatellite Analyzer: Dieringer and Schlotterer 2003).

Heterozygote deficiencies, once identified, can seemingly be corrected so as to prevent bias during genetic analysis. Such techniques fall into two broad classes: analytical or methodological. The former rely upon algorithms that quantify heterozygote deficiency, and subsequently rectify this bias by adjusting genetic data accordingly (Girard and Angers 2008). GenePop (Rousset 2008), for example, can estimate the overall frequency of null alleles based on heterozygote deficiencies, while MicroChecker (Van Oosterhout *et al.* 2004) can correct raw genotypes and account for the presence of null alleles based on their estimated frequencies. Other corrective measures have been recommended by Wagner *et al.* (2006), who proposed adjusting standard relatedness statistics to account for the possibility of null alleles. Programs like Cervus account for errors by incorporating a user-specified error rate for simulations of

parentage (Kalinowski *et al.* 2007). However, many of these analytical corrections have limited utility for downstream analysis of genetic patterns. Corrected genotypes for example cannot be used with multiple loci (Van Oosterhout *et al.* 2004) and software packages fail to accept allele frequency data, thus preventing analyses of null allele frequency estimates (Dewoody *et al.* 2006).

Methodological approaches, on the other hand, often involve use of alternative primers or adjustment of PCR conditions to boost the amplification of undetected alleles (Lehmann *et al.* 1996, Holm *et al.* 2001). This can require extensive laboratory effort and may not correct all null alleles (Walter and Epperson 2004), thus limiting information gain for effort invested. An alternative methodological path is the amplification of a greater number of polymorphic loci (Estoup *et al.* 2002), followed by the subsequent rejection of those possessing null alleles (Nascimento de Sousa *et al.* 2005). This approach can also be costly and time-consuming, particularly if the marker set contains multiple nulls. Moreover, this approach is relatively inefficient in the long run because it does not engage the underlying molecular issues *per se*.

Despite shortcomings, both analytical and methodological approaches can be employed to correct for the effects of null alleles. Yet remarkably, 90% of studies reporting null alleles failed to compensate for this error source and instead, researchers elected to employ the affected loci 'as is' (Dakin and Avise 2004). This is problematic in that null alleles are known to negatively impact analyses of genetic variability (Wagner *et al.* 2006), with the severity of bias depending on the type of analysis being performed. For example, F-statistics are particularly sensitive because null alleles can deflate within-population variance and result in exaggerated genetic differentiation (Chapuis and Estoup 2007). Simulations have shown that in parentage studies, average exclusion probabilities are reasonably immune to the effects of null alleles,

though their presence can significantly inflate the false exclusion rate for certain individuals (Dakin and Avise 2004).

Sources of genotyping error (Bonin *et al*. 2004) and methods to estimate error rate (Broquet and Petit. 2004; Creel *et al*. 2003) have been thoroughly addressed in the literature, mostly with simulation studies and theoretical approaches (Paetkau. 2003). A large number of publications focus on noninvasive sampling and its impact on error and population estimates (Lukacs and Burnham. 2005; McKelvey and Schwartz. 2004), because these studies are especially prone to genotyping error. The coverage of this topic has been so extensive that several protocols for noninvasive genotyping have been designed specifically for error reduction (Paetkau. 2003; Woods *et al*. 1999). However, genotyping errors are less commonly addressed in large-scale studies with robust tissue samples, and large DNA yields. Further, empirical reports of errors and their resulting effects on biological interpretations are relatively rare. Yet as microsatellites become increasingly popular in guiding management, conservation or disease detection (Moxon and Wills 1999), the need for evaluating errors becomes critical because consequences for biological misinterpretation could be severe.

In this study, we utilized microsatellite markers previously described for use in white-tailed deer (*Odocoileus virginianus*) (Anderson *et al.* 2002) to demonstrate the effects of null alleles on population genetics studies and the benefits that can be gained from their correction. These microsatellites are of particular interest because many have been used in prior genetic studies of hunter-harvested species, with the intent to guide wildlife and infectious disease management (DeYoung *et al.* 2003a, Blanchong *et al.* 2008). We applied empirical data to 1) describe genotyping errors found during large-scale genetic analysis and dissect their molecular basis, 2) compare methodological and analytical techniques most frequently used to rectify null

alleles, 3) determine how null alleles can impact biological interpretations and 4) provide recommendations on how genotyping errors and bias in population studies can be minimized.

Our study will improve the application of microsatellite technology for biological inference. By describing unreported null alleles and providing suggestions to reduce their bias we enable an increase in the efficiency of microsatellite analysis and demonstrate the need for thorough quality control measures in population genetics studies.

Materials and Methods

Laboratory Procedures

White-tailed deer (*N*=877) sampled through Illinois Department of Natural Resources (IDNR) Chronic Wasting Disease (CWD) surveillance in north-central Illinois were used for genetic analyses. Skeletal muscle and lymph node tissues of sampled animals were stored in 100% ethanol or in a -80°C freezer. Genomic DNA was extracted from these tissues using the Wizard Genomic DNA Purification Kit (Promega, Madison WI) in accordance with manufacturer's instructions. Individuals were genotyped using 13 microsatellite primers previously developed for white-tailed deer (Anderson *et al.* 2002, Blanchong 2003). This panel included markers BM848, BM1225, BM4107, BM6506, CSN3, (Bishop *et al.* 1994), BM4208 (Talbot *et al.* 1996), IGF-1 (Kirkpatrick 1992), OBCAM (Fries *et al.* 1993), OarFcb304 (Buchanan *et al.* 1993), RT20, RT23, RT27 (Wilson *et al.* 1997) and Srcrsp-10 (Bhebhe *et al.* 1994). Forward primers were labeled with fluorescent dyes (NED, HEX, FAM) and fragments were separated on an ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA) and visualized with GeneMapper (v. 4.0; Applied Biosystems, Foster City, CA).

Detection of Genotyping Errors

MicroChecker (v.2.2.3; Van Oosterhout *et al.* 2004) was used to test for genotyping errors, while GenePop (v.4.0; Rousset 2008) was applied to examine deviations from HWE. Observed (H_o) and expected (H_e) heterozygosities were calculated using 10,000 dememorization steps and 10,000 iterations in each of 100 batch runs with significance (P<0.01) based on the Markov chain algorithm (Rousset 2008). To resolve mismatched genotypes and their sources, 86 individuals were genotyped in replicates of 2 or 3 using all markers. Further, 35 female deer with twins or triplets *in utero* (fetuses N=65) were genotyped as mother-offspring pairs to identify mismatches resulting from null alleles overlooked in samples lacking pedigree information. Mismatches among replicates and mother-offspring pairs were counted as the number of genotypes with at least one erroneous allele. To calculate error rates, the number of mismatches was divided by the total number of genotypes for each locus.

Error sources were classified into four categories: null alleles, miscalls, transcription errors and allelic dropout. Null alleles were considered the source of all homozygote-heterozygote mismatches in markers that deviated from HWE or had nulls confirmed though sequencing (methods described below). Miscalls were considered the error source for genotypes that were incorrectly scored upon first visual inspection (usually when aberrant stutter patterns or spurious peaks were present). Transcription errors resulted from typing errors during manual data editing and allelic dropout was considered the source of error for genotypes scored heterozygous and homozygous in replicate reactions.

Effects of Sample Size

To determine if sample size affected our ability to detect deviations from HWE, subsets of 25, 50, 100, 200, and 400 individuals were randomly selected for analysis and compared with the

full data-set (722 deer genotyped; 877 total deer with 65 fetuses and 90 replicates removed). For each of these, F_{IS} was calculated and significance ($P<0.01$) evaluated with GenePop (v.4.0) for all loci using 10,000 dememorization steps and 10,000 iterations in each of 100 batch runs.

Methodological Corrections

When null alleles were detected, their molecular basis was investigated by direct sequencing of microsatellite repeat and flanking regions. Each locus was amplified via PCR, and products purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing was performed using a BigDye Terminator Sequencing kit and an ABI 3730XL DNA Sequencer (Applied Biosystems). Chromatograms were evaluated with CodonCode Aligner software (v. 3.5.1 CodonCode Corporation, Dedham, MA) and only nucleotide sequences possessing Phred quality scores of 20 or higher were considered for analysis. Upon detection of mutations in microsatellite flanking regions, primer binding sites were relocated to areas of higher fidelity: CSN3 (original reverse primer CSN3-R: GCACTTTATAAGCACCACAGC; re-designed reverse primer CSN3-R_{RD}: TAGCTCATAATGTAAACCACTTT) and locus RT20 (original forward primer RT20-F: GCAGAAGAGTGAGTGTGAGT; re-designed RT20-F_{RD} forward primer: TGGAAGATTTCAGAAATGAT).

Genotypes from redesigned primers were compared with raw genotypes to determine n_0, the number of heterozygous genotypes that were incorrectly scored as homozygous. Empirical estimates of null allele frequencies (*r*) were calculated by dividing n_0 by the total number of alleles analyzed for each locus.

Analytical Corrections

Four algorithms for estimating null allele frequencies (*r*) were compared across the five loci that consistently deviated from HWE. MicroChecker was used to calculate null allele frequencies

using analytical corrections described by Oosterhout *et al.* (2004; AC-O), Chakraborty *et al.* (1992; AC-C), Brookfield (1996; equation 1; AC-B). GenePop was employed to calculate null allele frequencies according to analytical methods described by Dempster *et al.* (1977; AC-D). MicroChecker was used to identify homozygous genotypes likely to be heterozygous for a null allele based on deviations from HWE. Corrected genotypes were calculated for locus BM848 and compared to original genotypes to determine, n_0, the number of heterozygous genotypes that were incorrectly scored as homozygous. Because MicroChecker does not perform genotype corrections for markers with null allele frequencies <5%, n_0 for locus BM4208 and locus BM6506 could not be calculated.

Evaluation of Corrective Measures

Date derived from five marker panels were compared to determine if analytical and methodological correction measures affected the outcome of analyses. Data sets were based on genotypes generated with: the original panel containing five loci with null alleles (DS-O), original panel with corrections for null alleles based on frequency estimates (DS-C), original panel with redesigned primers (DS-R), original panel with markers containing unresolvable nulls removed (DS-U) and original panel with redesigned primers added and markers containing unresolved nulls removed (DS-RU). Loci comprising each panel are described in Table 3.1. These five marker panels allowed us to compare data sets with equal numbers of loci and varying null allele frequencies.

When downstream analyses required corrected frequencies, the methods described by Brookfield *et al.* (1996; equation 1) were employed for null allele frequency estimation (as recommended by Van Oosterhout *et al.* 2004). Null allele frequencies (*r*) for each panel were

derived by summing the empirical estimates of null alleles for RT20 and CSN3 and calculated estimates for BM848, BM4208, and BM6506.

Corrective measures and population analyses

To examine population differentiation, sampled animals were divided among 26 study sites (with approximately equal numbers of deer within each site). Problematic markers BM4208, BM6506, BM848, RT20, and CSN3 were evaluated individually to determine the response of each marker to corrective measures. Pairwise F_{ST} values were calculated for all study site combinations using allele frequencies from original genotypes, corrected frequencies, and frequencies generated with redesigned primers. To further evaluate differences between these markers, Fisher's exact test for population differentiation was performed in Arlequin (v. 3.1; Excoffier *et al.* 2005) by using 100,000 Markov chain steps with 10,000 dememorization steps at a significance level of 0.05. The total number of significantly different study sites was summed for each data set and used as a metric for overall population differentiation.

To ensure that population differences were not solely attributable to variable explanatory power between data sets, POWSIM (Ryman and Palm 2006) was used to estimate the power to detect differentiation for each locus considered. For power analyses, Fisher's exact tests were performed on allele frequencies using 1000 dememorization steps followed by 1000 iterations in each of 100 batches. Power was calculated as the number of significant tests per 1000 replicates.

Effects of null alleles on population-based distance measures and spatial autocorrelation analyses were examined with Spatial Genetic Software (v. 1.0; Degen *et al.* 2001). This program derives calculations from allele frequencies rather than individual genotypes, thus permitting an evaluation of analytical corrections for null alleles. Genetic distance matrices were derived for all five data sets using either F_{ST} (Pons and Petit 1995) or Nei's genetic distance (Nei 1972) among

the 26 populations, while a geographic distance matrix was computed from the centroid of each study sited based on x- and y- coordinates. To test for spatial autocorrelation, average genetic distances were calculated within nine distance classes and compared to estimates of genetic distance averaged across all classes. Equidistant intervals were chosen so that each distance class had at least 30 data points and significance was evaluated using 500 random permutations of the observed data (Degen *et al.* 2001).

Corrective Measure and Parentage

To determine the effects of null alleles on parentage studies, Cervus (v. 3.0; Kalinowski *et al.* 2007) was used to assign parentage to 65 deer fetuses. Thirty-five confirmed mothers and 35 randomly selected females were included as candidate mothers to explore changes in exclusion probabilities in the presence of null alleles. Parentage assignments were based on data from four (DS-O, DS-R, DS-U, and DS-RU) of the five marker panels because Cervus cannot deal with frequency data (i.e. DS-C). To determine the maximum false exclusion rate, initial simulations were run with the proportion of loci mistyped set to 0. However, in subsequent simulations the proportion of loci mistyped was adjusted to account for panel specific error rates, as recommended by Kalinowski *et al.* (2007). For each panel, false exclusion rates were calculated by dividing the number of unassigned fetuses by the total number of confirmed mother-fetus pairs. Average non-exclusion probabilities were calculated for all loci combined within each simulation according to Kalinowski *et al*. (2007).

Results

Null Alleles and Genotyping Errors

Out of the original 13 microsatellites evaluated in this study, six deviated from HWE and one amplified inconsistently when the original panel was evaluated. Of the six markers that deviated

from HWE, RT23 and RT27 did not appear to be affected by null alleles (discussed below), whereas null alleles were confirmed for BM848, BM6506 and RT20. The source of HWE deficiency could not be resolved for the sixth marker (BM4208). In addition CSN3, which did deviate from HWE but amplified inconsistently, contained a confirmed null allele.

Direct sequencing of microsatellite loci showed that null alleles detected in RT20 resulted from duplications in the 5' microsatellite flanking region. Sequencing also indicated that BM848 and BM6506 contained mutations within the microsatellite repeat sequence (=imperfect repeat), and CSN3 had an insertion and two point mutations in the 3' flanking region (Fig. 3.1). Though loci RT23, and RT27 deviated from HWE in the full data set, F_{IS} values were extremely low (Table 3.2) and these deviations were not apparent in data subsets, nor larger data sets (data not shown), most likely suggesting subpopulation structure rather than null alleles. Interestingly, null alleles were detected in primers originally designed for cattle, (BM848, BM6506 and CSN3) and caribou (RT20).

Among the five data sets derived from different marker panels, overall frequency of null alleles (*r*) (calculated empirically for RT20 and CSN3 and estimated by Brookfield 1996; equation 1 for BM848, BM4208, and BM6506) ranged from 25% with the original panel (DS-O) to 0% in the original panel with redesigned primers minus unresolved nulls (DS-RU) (Table 3.1). Null alleles were the main source of errors (58%) in mother-fetus and replicate data sets, though miscalls, transcription errors and allelic dropout also contributed to mismatched genotypes (Fig. 3.2). Overall error rates for individual markers ranged from 0% (Srcrsp-10 and $CSN3_R$) to 4.5% (RT20) with an average error rate of 1.1% (Table 3.3).

Effects of Sample Size

The ability to detect HWE deviations was profoundly influenced by the sample size of the data set (Table 3.2). Eight of 15 (13 original+two redesigned) markers experienced changes in significance level when varying numbers of individuals were included in the analysis. Here, two lost and six gained significance. For markers BM848, BM6506 and RT20, F_{IS} and its associated significance increased as individuals were added to the data set. For marker BM6506, 400 individuals were analyzed before a significant departure from HWE was observed. In contrast, F_{IS} and its significance decreased for BM4107 and IGF-1 as sample size increased. Though significance changed for markers RT27 and RT23, F_{IS} values were generally low, unstable and with significant departures for HWE detected only in the full data set.

Methodological Corrections

After identifying null alleles in RT20 and CSN3, primers were redesigned to bind with greater fidelity. These redesigned primers consistently amplified alleles, with resulting genotypes in HWE. Amplification of BM4208 under original conditions produced spurious bands that often masked true microsatellite alleles. This problem was resolved by decreasing the number of PCR cycles from 25 to 20, so as to prevent PCR products from self-annealing (Bovo *et al.* 1999). Though spurious bands were eliminated, subsequent deviations from HWE were still apparent in this marker. Direct sequencing failed to reveal mutations in either the flanking or repeat regions, and the source of homozygote excess remains unresolved.

Analytical Corrections

The four analytical methods to estimate null allele frequency produced identical results for BM4208 (r=0.04) and BM6506 (r=0.01) (Table 3.4). For BM848, estimates based on AC-O, AC-B, and AC-D were identical (r=0.05), while values for AC-C were slightly higher (r=0.06).

Empirical null allele estimates (determined from genotypes that went from homozygous to heterozygous with redesign primers) were 0.02 for CSN3 and 0.13 for RT20. In comparison, methods AC-O and AC-C correctly estimated the frequency of null alleles for CSN3, while methods AC-B and AC-D produced slight underestimates (r=0.01). For RT20, only method AC-B accurately estimated the null allele frequency, while the other three methods produced overestimates. Based on these results, the method AC-B (Brookfield 1996) produced the most accurate estimates of null alleles in our study.

Corrective Measures and Population Analyses

For all five markers tested, average pairwise F_{ST} values for deer in 26 study sites were higher when calculated with null alleles. This overestimation was so severe for RT20 that the distributions of F_{ST} values for nulls compared to corrected and redesigned frequencies was significantly higher (F=2.9; P=0.057). The use of corrected null allele frequencies appeared to rectify this problem as average F_{ST} values for corrected and redesigned data sets were nearly identical. As illustrated by Fig. 3.3, when the number of different study sites was used as a metric for population differentiation, variable results were observed for all loci examined. Differentiation was higher for BM4208 and RT20 when nulls were included as compared to corrected and redesigned frequencies (Fig. 3.3). In contrast, markers BM6506, CSN3 and BM848 had higher levels of population differentiation with corrected frequencies compared to original frequencies including null alleles. The differences in population differentiation are attributable to null alleles rather than variations in genetic resolution, as changes in power averaged less than 2% among data sets for the same locus (Fig. 3.3).

Though population differentiation was sensitive to null alleles, population-based measures and spatial autocorrelation were only slightly affected by their presence. Overall

patterns of spatial autocorrelation were unchanged between data sets, as all showed significant positive autocorrelation within the first and seventh distance classes. However, average F_{ST} values and Nei's genetic distances were variable for data sets with equal numbers of loci. Based on 13 loci, data set DS-C yielded the highest distance values, followed by DS-O and DS-R. For data sets based on 10 loci, genetic distances were higher for DS-U than for DS-RU. To demonstrate the differences among data sets, simple linear regression was used to show Nei's genetic distances at each distance interval (Fig. 3.5).

Corrective Measures and Parentage Analysis

Corrective measures were also evaluated using parentage analyses. Parentage assignments for known mother (35) and fetus (65) pairs revealed that false exclusion rates increased drastically as the frequency of null alleles increased within data sets (Fig. 3.4). The original data set (r=25%) had the highest false exclusion rate (20%) with 13 of 65 confirmed mothers being excluded due to mismatches with their fetuses. On the other hand, the original data set with redesigned markers minus unresolved nulls (r=0%) did not falsely exclude any confirmed mothers due to mismatches. There also appeared to be an inverse relationship between the frequency of null alleles within a data set and the probability of non-exclusion. The original data set with redesigned markers minus unresolved nulls had the highest probability of non-exclusion (P=0.004) while the original data set had a much lower probability of non-exclusion (P=0.0003). However, these changes were mostly attributable to higher resolving power and not solely to null alleles, as data sets with equal numbers of loci (original vs. original with redesigned, original minus unresolved nulls vs. original with redesigned minus unresolved nulls) had similar estimates of probabilities of non-exclusion (<1% change in P).

Panel-specific error rates (calculated from mismatches in replicate samples and from mother-fetus pairs) were specified for parentage analyses in Cervus (Kalinowski *et al.* 2007), though they failed to rectify problems with false exclusion for genotypes based on the original data set. The proportion of loci mistyped was set to 1.3% for the DS-O, yet 13/65 confirmed mothers (20%) were still excluded. On the other hand, genotypes based on the other three data sets did not falsely exclude any confirmed mothers when panel specific error rates were used (0.5% DS-RU, 1% for DS-U and 1.1% for DS-R).

Discussion

Microsatellite markers have truly changed population studies by providing a wealth of genetic information for conservation biology, molecular ecology and population genetics. These disciplines, among many others, have benefitted greatly because microsatellite analysis provides an efficient way of estimating complex population parameters like dispersal, kinship, inbreeding and population size. The ability of microsatellites to elucidate such intricate genetic patterns however, depends greatly on the accuracy of genotypes.

Null Alleles and Genotyping Errors

Using empirical data, we were able to demonstrate that null alleles accounted for the vast majority of genotyping errors in our study. These loci are generally considered validated markers and extensively employed in population studies (Anderson *et al.* 2002, DeYoung *et al.* 2003b, Blanchong *et al.* 2008). The fact that >30% of our original panel contained confirmed null alleles suggests that more stringent criteria for marker evaluation need to be considered. The validation study of Anderson *et al.* (2002) included markers BM848, BM6506, and BM4208 and while $H_O<H_E$ for all three, the authors conclude that "little evidence exists of null alleles at these loci," with a recommendation for use in studies of gene flow and parentage (Anderson *et al.* 2002).

Clearly, our results reiterate the need for a thorough marker assessment with each novel application, as microsatellites that are appropriate for one study may be problematic for alternative populations, even within the same species.

Effects of Sample Size

Elevated sample sizes greatly enhanced our ability to detect low frequency null alleles and allowed us to examine the effects of sample size on the detection of HWE deviations. Sample size issues are a consistent problem for ecological studies (B-Rao. 2001; Muirhead *et al*. 2008), especially those involving rare or endangered species (Storfer 1996). Our results suggested that elevated samples sizes are necessary to detect markers with null allele frequencies ≤5%. BM848 required N=200 and BM6506 required N=400 before HWE deviations were significant, contrary to suggestions of DeYoung *et al.* (2003b) that 50 individuals are sufficient for estimating population allele frequencies. Simulation studies have supported our results by demonstrating that sample sizes <100 are unlikely to produce reliable estimates of population allele frequencies (B-Rao. 2001). Moreover, HWE inconsistencies observed during subsampling show that dividing populations into smaller groups for analysis of HWE (as suggested in MicroChecker for N>750; Van Oosterhout *et al.* 2004) can lead to erroneous conclusions about population-level deviations, and this suggestion should be considered when evaluating null alleles in large data sets.

Methodological Correction

In general we found that sequencing the primer-binding sites of markers suspected to contain null alleles was an effective way to resolve the molecular basis for deviations from HWE. Further, information about the molecular origins of null alleles can be useful for determining which type of corrective measures will best rectify the problem. For example, duplications in the

primer binding site of RT20 produced multiple fragments corresponding to the same allele, and corrected genotypes based on estimated null frequencies were unable to compensate for the complexity of this issue. Redesigning the primer, on the other hand, resulted in HWE and a drastic decrease in population differentiation as compared to full and corrected frequencies. By understanding the molecular basis of null alleles and the methods used to estimate their frequencies, one can *a priori* predict the utility of analytical corrections. In general, the cost of sequencing a few individuals is much lower than the cost of testing additional markers, thus sequencing is an excellent method for exploring null alleles.

Corrective Measures and Population Analyses

Null alleles bias allele frequencies by overestimating observable alleles, thereby decreasing observed heterozygosity and increasing the apparent level of inbreeding (Dewoody *et al.* 2006). This leads to overestimation of population differentiation (Chapuis and Estoup 2007), as was observed for markers RT20 and BM4208. For CSN3 however, population differentiation was lower for genotypes with null alleles as compared to genotypes from redesigned primers. Relocating primer binding sites promoted amplification of novel alleles at this locus which in turn, improved discriminatory power and resulted in greater differentiation. Because CSN3 is not very polymorphic (H_e=0.5) and null allele frequencies were low (2%), the discriminatory power from additional alleles was greater than diversity lost because of null alleles. Still, variation in population differentiation observed among all of our markers would have led to alternate conclusions about the intensity of gene flow among study sites. Collectively these findings suggest that null alleles can have variable influences on levels of population differentiation, depending on genetic diversity of the marker and the frequency of the null allele.

Despite their impacts on parentage and population differentiation, null alleles did not affect overall patterns of spatial autocorrelation. Nevertheless, differences in Nei's genetic distances and F_{ST} values were observed for panels with and without null alleles. As noted in other studies, analyses of population structure tend to be less sensitive to errors than individual-based analyses (Taberlet *et al.* 1999), and in our study this was most likely because bias inflicted by null alleles was diminished by the rest of the markers in the panel. This analysis is more realistic than our single-locus analysis of population differentiation because most population studies apply multilocus panels that are likely to contain combinations of markers with and without null alleles. The fact that spatial autocorrelation results were qualitatively similar suggests that null allele frequencies ≤25% will have minimal effects on overall patterns of population structure, though even at frequencies as low as 10% differences in distance measures will undoubtedly be affected.

Corrective Measures and Parentage Analysis

Consistent with simulations studies, false exclusion rates in parentage analyses for our study increased dramatically as the frequency of null alleles increased (Dakin and Avise 2004). Dakin and Avise (2004) recommend circumventing this problem by excluding candidate parents only if mismatches occur at >1 locus. Our results suggest that this *post hoc* approach may be too conservative for preventing false exclusions, as we detected null alleles in almost 40% of the markers employed, and mismatches across multiple loci were detected for mother-fetus pairs. An alternative correction for null alleles in parentage analysis is to account for genotyping errors during parentage assignment (Kalinowski *et al.* 2007). By specifying a user-defined error rate, Kalinowski et al (2007) concluded that the number of false exclusions could be reduced. While defining an estimated error rate was able to improve false exclusions in data sets with null allele

frequencies ≤15%, it failed to decrease false exclusions when null allele frequencies were 25%. This is most likely because null alleles tend to violate Kalinowski *et al.*'s (2007) assumptions of random erroneous genotypes and equal error rates for all loci, but it could be because error rates in this study were underestimated. Some studies have concluded when frequencies of null allele are low (<20%) their effects on parentage are minimal (Dakin and Avise 2004). While this was confirmed in our study in regards to exclusion probabilities, low frequency null alleles (<15%) still yielded appreciable false exclusion probabilities. In general, null alleles can hinder parentage analyses, and analytical measures to correct for null alleles do not always remedy their negative effects.

Collectively our findings emphasize the need for consistent evaluation of genotyping error throughout all stages of population studies. Researchers cannot solely rely on validation and simulation studies to provide recommendations for certain microsatellite markers. The utility of a marker panel is dependent on the research objectives at hand and the effects of null alleles are far too stochastic to make generalized statements about their pervasiveness as well as the appropriateness of corrective measures. With that said, we believe there are several ways that researchers can prevent genotyping errors and subsequent bias in biological interpretations of genetic data.

1) Clearly define study objectives: This will dictate the level of genetic resolution needed and thus the number of microsatellites that will be required. We found that simulation programs like POWSIM (Ryman and Palm 2006) can be extremely helpful in guiding marker selection because they allow the user to determine the number of marker and polymorphism level needed to detect genetic differentiation. Furthermore, Genetix (Belkhir *et al.* 2004) allows users to simulate interpopulation dispersal under alternate models of mutation and genetic resolution, and

this can be used to explore the effects of marker diversity on assignment tests. By exploring different scenarios of genetic exchange for the target population, the number of markers and level of polymorphism needed to address specific research objectives can be estimated *a priori*.

2) Choosing markers: After defining study objectives, choose a panel of candidate markers that was developed for the target species, or a phylogenetically-close relative, as decreasing phylogenetic distance generally decreases the probability of null alleles (Chapuis and Estoup 2007). However, our data warrant caution as markers designed for cattle (*Bos Taurus;* suborder Ruminantia, family Bovidae; BM848, BM4208, BM6506) had lower frequencies of null alleles in white-tailed deer than did markers designed for caribou (*Rangifer tarandus*; suborder Ruminantia, family Cervidae; RT20) despite the latter belonging to the same taxonomic family. In selecting markers, prepare for the possibility of non-amplification, monomorphism or severe null alleles by testing a surplus of candidate markers. By doing so, poor performing markers can be eliminated early on and resources can be allocated towards optimizing markers with higher potential for success.

3) Microsatellite Optimization: Testing markers on individuals from different sampled populations or genetic cohorts will enhance the potential for determining polymorphism for the target population. Following PCR, the fragment size should be compared to standards from the literature to prevent amplification of non-specific products resulting from multiple primer binding sites. During optimization, it is helpful to determine an optimal DNA concentration as this can vary drastically for different microsatellites. Many studies report an increase in genotyping errors as DNA quantity and quality decrease (Gagneux *et al.* 1997a, Taberlet *et al.* 1999). DNA extracted from feces (Bellemain *et al*. 2005), hair (Taberlet *et al*. 1996), and museum specimens (Horvath *et al*. 2005), can be extremely difficult to genotype and tends to

produce genotypes that are unreliable and error prone. Our own experience suggests that DNA from formalin fixed samples or improperly preserved tissues had extremely high failure and error rates because of inferior template quality, and we recommend avoiding these types of samples. In addition, ensure reliable and repeatable amplification for all individuals. To assume that laboratory errors are the cause of non-amplification could lead to the inclusion of null alleles in the data set.

4) Pilot Study: These can greatly reduce potential losses from genotyping errors by providing an opportunity to quantify errors, identify their causes and eliminate their sources early on. Independent replication of at least 5-10% of samples is recommended for assessment of marker-specific error rates (Pompanon *et al.* 2005), and if possible the inclusion of samples with known pedigrees can greatly facilitate the identification of invisible null alleles. During a pilot study, an ample number of individuals must be analyzed so that marker-specific stutter patterns, size range, and aberrant bands can be identified. Throughout this process, pay attention to spurious bands or inconsistent stutter patterns as these artifacts can be indicative of inefficient optimization (Bovo *et al.* 1999) or adjacent allele heterozygotes. To prevent downstream difficulties, we recommend discarding any microsatellites that unreliably amplify, consistently deviate from HWE or produce genotypes that are difficult to score.

5) Comprehensive Study: Our findings reiterate the need to evaluate errors for the entire duration of the genotyping process. Software packages like MicroChecker (Van Oosterhout *et al.* 2004) should be continually employed to check for HWE and null alleles, even as additional samples are genotyped. Independent replicate genotyping should also be conducted throughout the study so that error rates can be assessed during all steps of sample processing. If null alleles are suspected, then estimating their frequency can be helpful in determining the utility of the

marker for various genetic analyses, but it should not be used exclusively to determine the level of bias that the null alleles will impart.

Our study provides empirical insight into the unpredictable nature of genotyping errors and their effects on measures of population structure and parentage. We detected several null alleles in microsatellites that were developed and oft employed in managed species and we show that these nulls can introduce substantial bias in results. For our study, the inclusion of null alleles would have led us to conclude that populations were more genetically distinct than they truly were. Further, we would have been unable to determine paternity for a large proportion of samples, despite the fact that true parents were included. Biased conclusions such as these could lead to the false identification of management units, or inferences about breeding systems that are inaccurate. Others wishing to employ this genetic technology should note that analytical approaches to correct for null alleles were unable to fully compensate for their bias. Thus caution should be exercised when these techniques are employed. Our results demonstrate that genotyping errors are study-specific and additional research is needed to develop standardized error detection protocols and further evaluate corrective measures. We believe that standards for genotyping would limit errors and result in a higher level of accuracy in population studies, making biological interpretations more meaningful. Though the microsatellites evaluated in this study were designed for white-tailed deer we feel that our recommendations are applicable to anyone employing these genetic markers.

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Table 3.1. Microsatellite marker panels used for genetic analysis of null alleles.

	Marker Panel (Data Seta)[a]				
Marker	Original (DS-O)	With Corrections[b] based on null frequency estimates (DS-C)	With Redesigned (DS-R)	Original minus unresolved nulls (DS-U)	With Redesigned primers minus unresolved nulls (DS-RU)
BM1225	√	√	√	√	√
BM4107	√	√	√	√	√
BM4208	√	√$^{\beta}$	√		
BM6506	√	√$^{\beta}$	√		
BM848	√	√$^{\beta}$	√		
CSN3	√	√$^{\beta}$		√	
CSN3$_{\mathbf{R}}$			√		√
IGF-1	√	√	√	√	√
OarFcb304	√	√	√	√	√
OBCAM	√	√	√	√	√
RT20	√	√$^{\beta}$		√	
RT20$_{\mathbf{R}}$			√		√
RT23	√	√	√	√	√
RT27	√	√	√	√	√
Srcrsp-10	√	√	√	√	√
Total	**13**	**13**	**13**	**10**	**10**
r	25%	0%	15%	10%	0%

[a]Data sets derived from each marker panel are denoted in parentheses. [b]Indicates use of corrected frequency as calculated per Brookfield *et al.* 1996; equation 1. A √ indicates inclusion in panel. Rows with no shading indicate well behaved primers with no correction or redesign. Light gray shading indicates markers with potential null alleles or erratic amplification. Dark gray shading indicates redesigned primers. *r* = panel-specific estimate of total null allele frequencies (empirical *r* used for RT20, CSN3, $r^{AC\text{-}B}$ used for BM848, BM6506, and BM4208).

Table 3.2. Effects of sample size on null allele detection.

	N^1=25		*N*=50		*N*=100		*N*=200		*N*=400		*N*=Full (722)	
Marker	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*	F_{IS}	*P*
BM848	0.07	ns	0.05	ns	0.01	ns	0.07	**	0.1	***	0.11	***
BM1225	-0.02	ns	0.02	ns	0.02	ns	-0.02	ns	0.0	ns	0.01	ns
BM4107	0.24	*	0.12	ns	0.04	ns	0.05	ns	0.0	ns	0.03	ns
BM4208	0.20	**	0.13	**	0.06	*	0.07	***	0.1	***	0.07	***
BM6506	0.00	ns	-0.04	ns	-0.01	ns	0.03	ns	0.0	*	0.02	*
CSN3	0.07	ns	-0.08	ns	-0.10	ns	-0.13	ns	0.0	ns	0.04	ns
$CSN3_R$	-0.03	ns	-0.16	ns	-0.19	ns	-0.15	ns	0.0	ns	0.03	ns
IGF-1	0.22	*	0.13	ns	0.08	ns	0.04	ns	0.0	ns	0.01	ns
OarFcb304	-0.12	ns	-0.09	ns	0.04	ns	0.01	ns	0.0	ns	0.03	ns
OBCAM	-0.05	ns	0.01	ns	-0.01	ns	-0.05	ns	0.0	ns	-0.01	ns
RT20	0.27	**	0.27	***	0.28	***	0.26	***	0.3	***	0.29	***
$RT20_R$	0.00	ns	-0.02	ns	0.01	ns	-0.02	ns	0.0	ns	-0.02	ns
RT27	0.04	ns	0.01	ns	0.04	ns	-0.01	ns	0.0	ns	0.01	*
RT23	-0.10	ns	0.00	ns	0.00	ns	-0.02	ns	0.0	ns	0.01	***
Srcrsp-10	0.05	ns	0.06	ns	0.02	ns	-0.04	ns	0.0	ns	0.01	ns

[1]Subsets of 25, 50, 100, 200, and 400 individuals were randomly selected for analysis and compared with the full data-set (722 deer genotyped).

ns= P>0.05; * P<0.05; **P<0.01; ***P<0.001. Tests for heterozygote deficiency were performed and F_{IS} values were evaluated for significance using 10,000 dememorization steps followed by 10,000 iterations in each of 100 batches. Markers highlighted in grey demonstrated changes in significance for heterozygote deficiency at different values of *N*.

Table 3.3. Mismatch errors caused by null alleles in known pedigree and mother-fetus and replicate genotypes of white-tailed deer.

Marker	Mismatch mother-fetus*	Mismatch Replicate**	Proportion mismatched
Srcrsp-10	0.0	0.0	0.0
$CSN3_{\mathbf{R}}$	0.0	0.0	0.0
CSN3	0.0	4.0	1.6
IGF-1	0.0	1.0	0.4
RT27	0.0	1.0	0.4
RT23	0.0	1.0	0.4
BM1225	0.0	1.0	0.4
OarFcb304	0.0	2.0	0.7
BM4107	0.0	2.0	0.7
OBCAM	0.0	3.0	1.1
BM848	3.0	0.0	1.2
BM6506	3.0	2.0	1.9
BM4208	3.0	5.0	3.1
RT20	7.0	4.0	4.5
$RT20_{\mathbf{R}}$	0.0	2.0	0.8

*Mismatched genotypes between mother-fetus pairs for 65 fetuses and 35 mothers.

**Mismatched genotypes for replicate samples from 86 individuals. Proportion mismatched was calculated by taking the total number of mismatched genotypes (mother-fetus and replicate) divided by the total number of genotypes.

Table 3.4. Descriptive statistics for white-tailed deer microsatellites with null alleles before and after primer re-design.

	Original Primers					Re-designed Primers			Null Allele Estimates				
Marker	N	n_0	H_o	H_e	*P* value	H_o	H_e	*P* value	Empirical	r^{AC-C}	r^{AC-B}	r^{AC-D}	r^{AC-O}
RT20	716	192	0.62	0.87	*	0.87	0.86	ns	0.13	0.17	0.13	0.14	0.14
CSN3	710	31	0.48	0.50	ns	0.49	0.50	ns	0.02	0.02	0.01	0.01	0.02
BM848	716	65	0.76	0.85	***	na	na	na	na	0.06	0.05	0.05	0.05
BM4208	716	nc	0.83	0.90	***	na	na	na	na	0.04	0.04	0.04	0.04
BM6506	716	nc	0.86	0.88	ns	na	na	na	na	0.01	0.01	0.01	0.01

N=number of genotypes, n_0=number of genotypes that contained null alleles. nc=not calculable. H_o =observed heterozygosity, H_e=expected heterozygosity calculated by GenePop and evaluated for significance using the Markov chain algorithm. ns=$P>0.05$; *=$P<0.05$; ***=$P<$ 0.001.Empirical null allele estimates (*r*) were calculated by taking the number of null alleles (determined from genotypes that went from homozygous to heterozygous with redesign) divided by the total number of alleles analyzed. r^{AC-C} = null allele estimate according to Chakraborty *et al.* (1992); r^{AC-B} = null allele estimate according to Brookfield (1996); r^{AC-D} = null allele frequency according to Dempster *et al.* (1977); and r^{AC-O} = null allele frequency according to Oosterhout *et al.* (2004).

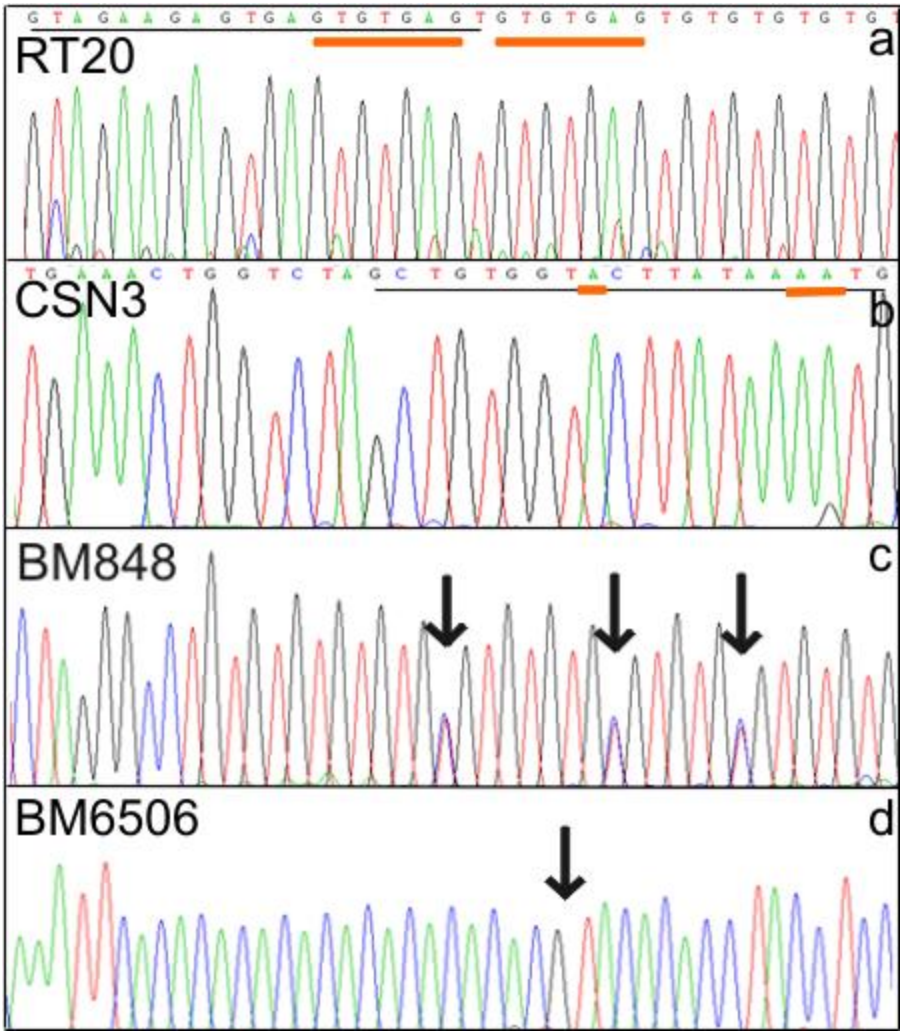

Figure 3.1. Molecular basis for null alleles detected in white-tailed deer microsatellites. Panel a, RT20 with original forward primer underlined in black (RT20 original forward primer: GCAGAAGAGTGAGTGTGAGT) and duplication in primer binding site underlined in orange. Panel b, CSN3 with original reverse primer binding site underlined in black (original reverse primer: GCACTTTATAAGCACCACAGC; reverse complement shown in chromatogram GCTGTGGTGCTTATAAAGTGC) with insertion and two base pair substitutions underlined in orange. Panel c, BM848 imperfect repeat with multiple base pair substitutions indicated by arrows. Panel d, BM6506 imperfect repeat with 'GT' insertion indicated by arrow.

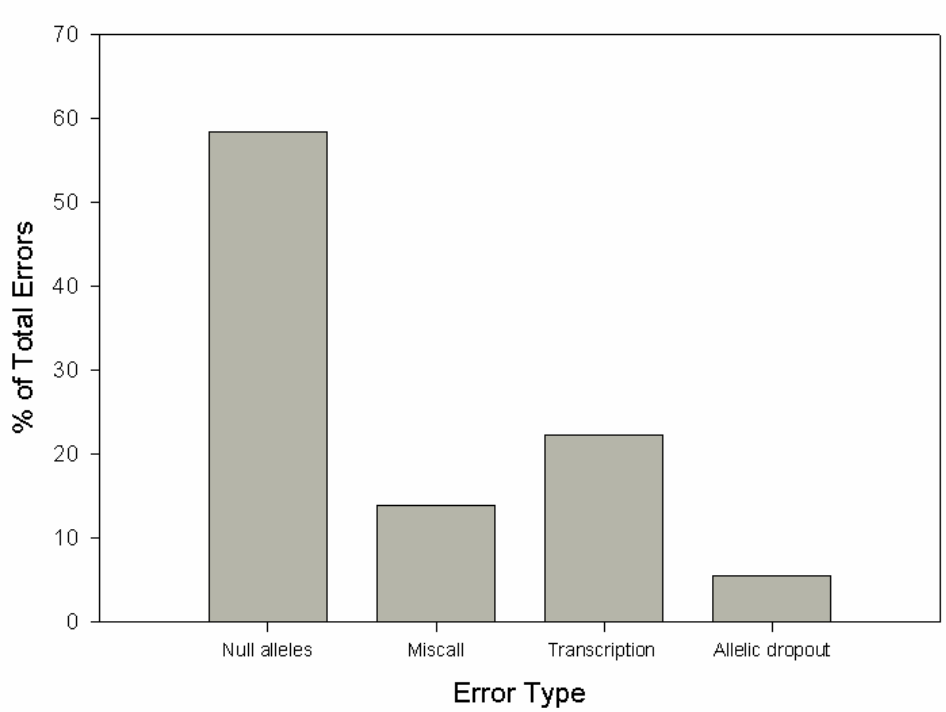

Figure 3.2. Types of genotyping errors detected in high throughput genetic analysis of white-tailed deer. Genotype mismatches were counted and characterized for mother-fetus pairs (65 fetuses and 35 mothers) and 86 individuals genotyped in replicate. The % of total errors was calculated as [(number of errors for each category/number of errors observed for all categories)*100].

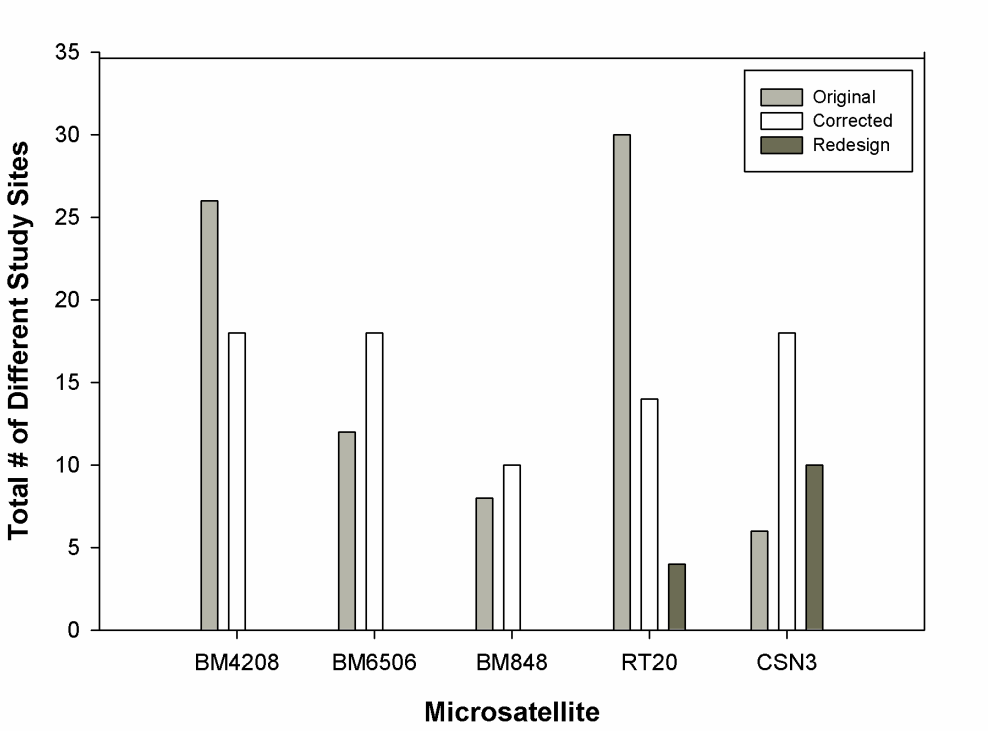

Figure 3.3. Effects of null alleles on estimation of population differentiation. Fisher's exact tests for population differentiation were performed using original, corrected and redesigned allele frequencies of individual microsatellites with 100,000 Markov chain steps and 10,000 dememorization steps in Arlequin. The number of different study sites at $P<0.05$ was summed and used as a metric for overall genetic differentiation. POWSIM was used to estimate the power to detect differentiation with Fisher's exact tests of allele frequencies. One thousand dememorization steps followed by 1000 iterations in each of 100 batches were used to determine significance for each test. Power was calculated as the number of significant tests per 1000 replicates, reported as a percent.

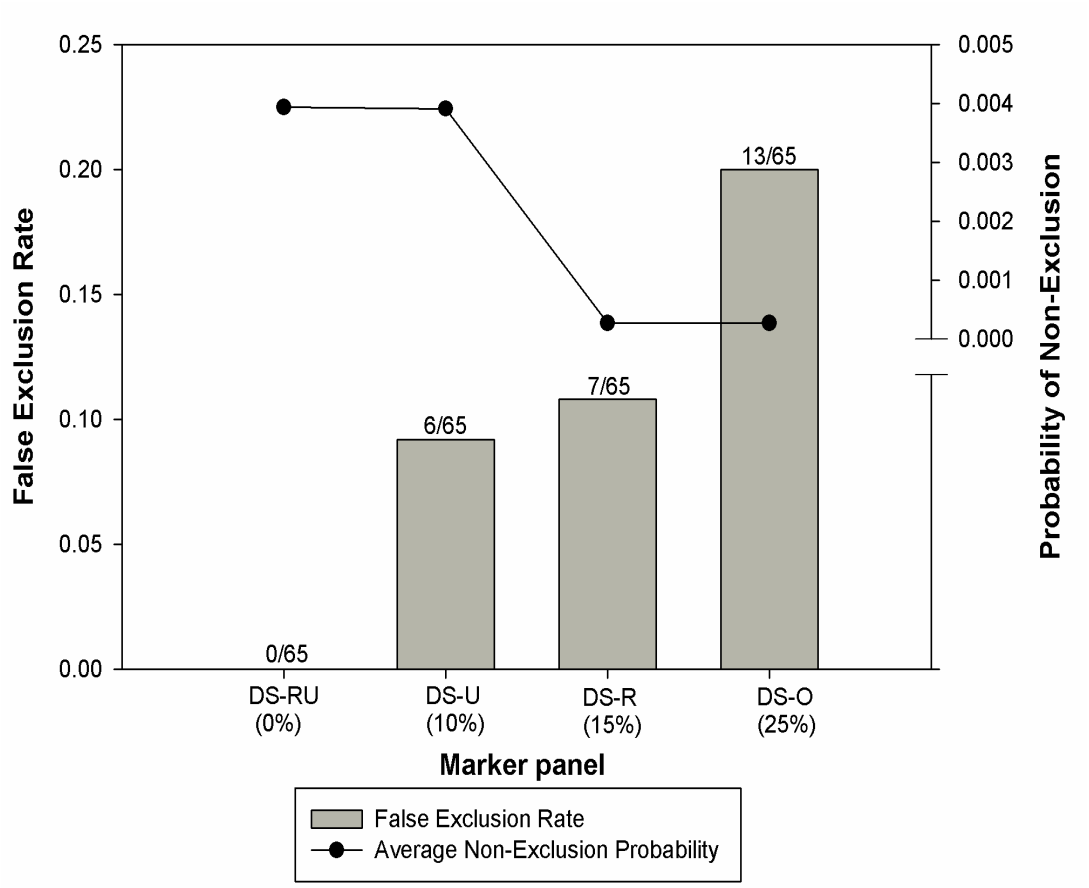

Figure 3.4. Effects of null alleles on parentage analysis. False exclusion rates and probabilities of non-exclusion for original (O), original with redesigned primers (O+R), original minus unresolved nulls (O-U) and original with redesigned primers minus unresolved nulls (O+R-U). Panel specific estimates of null allele frequency (*r*) are shown below each panel name. Parentage was assigned to 65 deer fetuses using 70 candidate mothers (35 known mothers and 35 random females). The proportion of individuals falsely excluded due to mismatches from null alleles is shown above each bar. Probabilities of non-exclusion were averaged across all loci and reported for each panel tested.

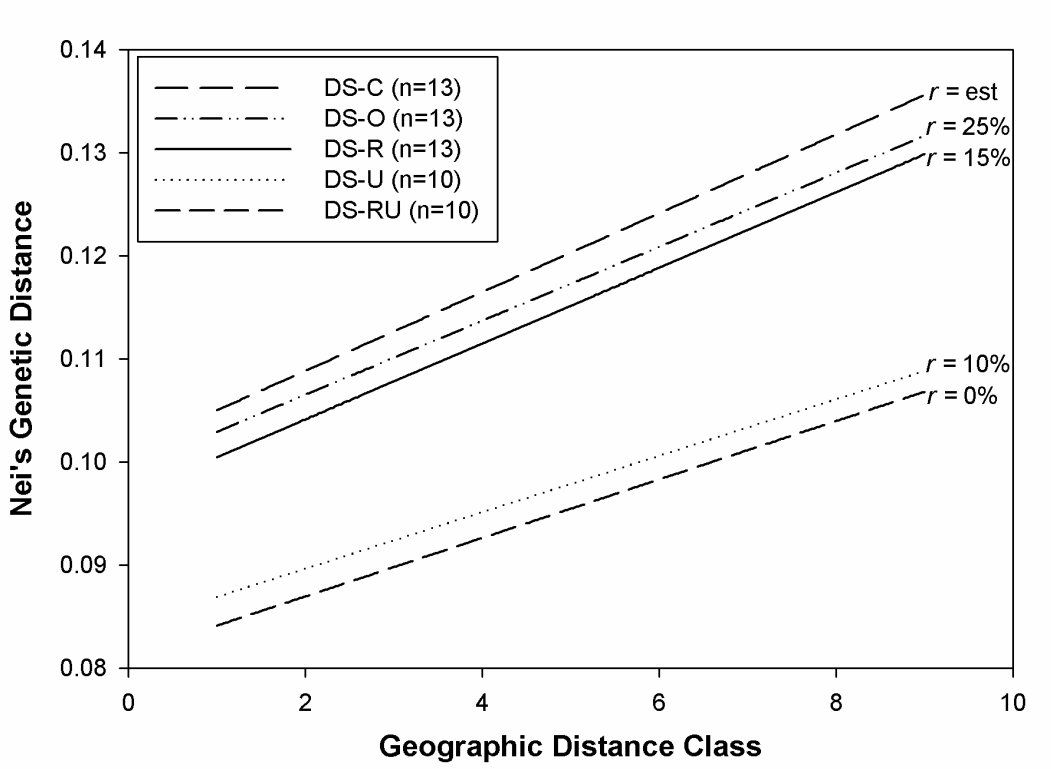

Figure 3.5. Effects of null alleles on genetic distance measures. Average Nei's genetic distances were calculated for 26 study sites within nine distance classes using five microsatellite panels: original with corrected frequencies (O+C), original (O), original with redesigned primers (O+R), original minus unresolved nulls (O-U), and original with redesigned primers minus unresolved nulls (O+R-U). Linear regression was used to show changes in average genetic distances for each geographic distance class. The frequency of null alleles (*r*) is shown to the right of each regression line.

Chapter 4: Genetic Characteristics of Managed White-tailed Deer Populations: Assessing the Impacts of CWD Surveillance

Abstract

The objective of this investigation was to use molecular genetic techniques to examine the consequences of chronic wasting disease (CWD) surveillance and population control in white-tailed deer herds. Chronic wasting disease management often involves decreasing deer densities to reduce the likelihood of disease occurrence and spread. Increased removal of individuals can alter genetic characteristics of the population, causing a loss of genetic diversity, a decrease in fitness, or enabling increased immigration. Molecular techniques can be used to evaluate genetic consequences and ecological implications of CWD control strategies, thereby promoting adaptive management. In this study, 10 selectively neutral microsatellites were examined in samples collected through disease and population control. Allele frequencies were compared among cohorts of deer to determine if culling changed the genetic composition of managed populations. Additionally, allele frequency distributions, heterozygosity, and genetic characteristics such as allelic richness and fixation indices were evaluated in pre- and post-cull deer populations to examine the effects of culling on effective population size, genetic differentiation or genetic diversity of white-tailed deer. Cohorts demonstrated little change in allele frequencies from year to year. However, evaluations of pre- and post-cull populations revealed increases in allelic richness and deficiencies in heterozygosity in post-cull populations, suggesting that these populations have received immigrants following intervention. Moreover, female deer, which tend to be philopatric, had significant changes in allele frequencies after culling was initiated. This study suggests that while reducing deer densities through culling enriches the genetic composition of deer, it could also result in immigration of CWD infected

deer, and these potential ecological consequences need to be considered during the implementation of disease management plans. This research contributes to the adaptive management of white-tailed deer in CWD infected regions and demonstrates the utility of genetic techniques as tools for evaluating wildlife management tactics.

Introduction

The discovery of chronic wasting disease (CWD) in free-ranging white-tailed deer drastically alters the focus of management for this big-game species within infected areas (Williams *et al.* 2002). In areas without CWD, herd management has generally been geared towards maintaining sustainable yields while minimizing land-use conflicts between deer and humans (Woolf and Roseberry 1998). However, in CWD infected areas, wildlife managers are forced to deal with a transmissible disease that has potential to spread to livestock or even humans. Of additional concern, CWD is a prion disease, so the disease causing agent has the potential to persist in the environment and cause subsequent infections (Miller *et al.* 2004; Johnson *et al.* 2006b). Disease control therefore, requires aggressive management which often involves herd culling to reduce deer density (Wobeser 2002). Reduced deer contact should limit further spread, and disease surveillance in culled deer can delimit the extent of the outbreak (Joly *et al.* 2003; Schauber and Woolf 2003; Joly *et al.* 2009). In addition, wildlife managers must also consider public perceptions of deer as a natural resource (Williams *et al.* 2002) because hunting provides economic benefits as well as a means for widespread population control (Schauber and Woolf 2003; Diefenbach *et al.* 2004).

Given the challenges of contemporary deer management, research in deer ecology and prion disease etiology can promote effective management and guide CWD surveillance. Furthermore, disease control strategies need to be evaluated, not only to monitor their success

(Wobeser 2002), but also to determine potential ecological consequences for the population. Extensive culling increases mortality, which can alter genetic characteristics of the population and result in an array of phenotypic responses (Allendorf *et al.* 2008). Reductions in effective populations size, or the number of breeding individuals, are commonly reported in over-harvested marine species (Hauser *et al.* 2002; Hutchings and Reynolds 2004), and a loss of genetic diversity has been documented for cervid populations that have undergone reductions (McCullough *et al.* 1996; Martinez *et al.* 2002; Nabata *et al.* 2004).

Culling can also increase gene flow into unoccupied niches which can disrupt population subdivision by decreasing genetic differentiation (Chesser 1991; Allendorf *et al.* 2008). This has been documented in red deer populations subjected to increased harvest, where increased male immigration resulted in low F_{ST} values and decreased population structure between culled locales (Nussey *et al.* 2006). While immigration may replenish some of the diversity lost during population reduction (Luikart *et al.* 1998), it can also dilute locally adapted alleles that provide a selective advantage. Hence, understanding the evolutionary and genetic consequences of culling would aid in adaptive management that limits reductions in diversity, fitness, or the loss of desireable phenotypes (Allendorf *et al.* 2008). Molecular genetic techniques provide an effective tool to examine how genetic characteristics respond to increased mortality (Schwartz *et al.* 2007). If genetic samples are collected before and after intervention, temporal comparisons of allele frequencies can quantify changes in effective population size and admixture that can occur after a population has been subjected to harvest. Further, monitoring genetic changes with selectively neutral markers is a powerful way to reveal recent changes in genetic diversity (Luikart *et al.* 1998) or cryptic patterns related to harvest that are not detectable through direct observation (Luikart *et al.* 1998; Schwartz *et al.* 2007; Allendorf *et al.* 2008).

Genetic techniques were applied to evaluate the effect of CWD control efforts in Illinois and Wisconsin. CWD was first reported in Wisconsin in February 2002 (Nolen 2002) and was reported in Illinois later that year (Illinois Department of Natural Resources 2010). Both states initiated management plans that involved regulating transport of captive cervids, testing hunter harvested deer for CWD, and culling areas with high risk of infection. In Wisconsin, the goal was to eradicate CWD in a 287 mi^2 area by removing as many deer as possible (Nolen 2002). In Illinois, however, CWD management was more focused, with culling efforts targeting areas within a one mile radius of CWD infected locations (Illinois Department of Natural Resources 2010). While CWD surveillance in Wisconsin has been able to reliably identify areas harboring positive cases (Joly *et al.* 2009), its effectiveness in terms of lowering prevalence or impacts on herd viability have yet to be fully assessed. In Illinois, culling appears to have kept prevalence low (Weng et al. 2010, in preparation), but the genetic impacts of CWD management still have not been described. The objective of this investigation was to use genetic metrics to examine potential genetic and ecological consequences of intensive CWD surveillance and population control. To address this issue, allele frequencies and rare alleles were examined among temporally-spaced cohorts and heterozygosity, allelic richness and fixation indices were evaluated in pre- and post-cull populations to determine if culling impacted effective population size, genetic differentiation or genetic diversity of white-tailed deer.

Materials and Methods

Deer Sampling

We utilized tissue samples collected through CWD surveillance and population control programs in central and northern Illinois as well as in southern Wisconsin. The majority of samples were collected from areas at increased risk for disease based on proximity to prior CWD cases (Figure

1) or from areas of special management interest. Harvest date, gender, age and spatial locations were collected for all samples, and every deer was tested for CWD using immunohistochemisty. For temporal analyses, deer were placed into cohorts according to birth year (aged using dentition), and also by birth year and sex. Cohorts were then assigned to pre- and post-cull populations for further evaluation. The spatial distribution of samples for each cohort is shown in Fig. 4.2.

North-central Illinois sampling area (NIL). Between January 2003 and March 2008, Illinois Department of Natural Resources (IDNR) conducted intense culling of free-ranging deer in the CWD-infected region of northern Illinois (Fig. 4.1). Of the ~5,000 samples collected, 920 from Winnebago, Boone, DeKalb, Ogle, and McHenry counties were used for genetic analysis. For deer sampled in NIL, spatial locations were recorded to the nearest township/range/section (TRS; 2.6 km^2). Deer sampled in NIL were born between 1998 and 2008, but only seven deer were sampled from 1998, so these deer were combined with the 1999 cohort. Likewise in 2007 and 2008, only 12 deer were sampled so these were combined with the 2006 cohort. Culling in Illinois began in 2002, thus deer born ≥2002 were assigned to the pre-cull populations and deer born after 2002 were assigned to the post-cull population.

East-central Illinois sampling area (RAP). During fall hunting seasons between 2005 and 2007, 277 free-ranging deer were harvested at University of Illinois Robert Allerton Park (RAP) through their Deer Management and Research Program (Fig. 4.1). For deer sampled in RAP, spatial locations were recorded to the nearest km. Deer sampled in RAP were born between 2001 and 2007, but only 3 deer were sampled in 2007 so these were combined into the 2006 cohort. Culling in RAP began in 2004 and so deer born ≥2004 were assigned to the pre-cull population

and deer born after 2004 were assigned to the post-population control cull population. At the time of this investigation, CWD had not been detected in the park.

Wisconsin sampling Area (WI). Between October 2003 and March 2006, Wisconsin Department of Natural Resources (WDNR) collected samples from 71,611 free-ranging deer harvested by hunters in the CWD management zone in southern Wisconsin. Of the samples collected, 479 from Grant, Lafayette, Green, Rock, Walworth, Racine, and Kenosha counties were used for genetic analysis (Fig. 4.1). For deer sampled in WI, spatial locations were recorded to the nearest township/range/section (TRS). Deer sampled in WI were born between 2000 and 2005, but only 2 deer were sampled in 2000, so these were combined with the 2001 cohort. Similarly birth year cohorts 2003 and 2004 were combined because of low sample number. Following CWD detection in WI, hunter harvest sample collection for disease surveillance began in 2002, thus deer born ≥2002 were assigned to the pre-hunter harvest cull population and deer born after 2002 were assigned to the post-hunter harvest cull population.

Laboratory Procedures

Muscle and lymph node samples were stored in 100% ethanol or frozen (-20) and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison WI). Individuals were genotyped using 10 microsatellite DNA primers previously described in Kelly et al. (2010). Forward primers were labeled with fluorescent dyes (NED, HEX, FAM) with fragments separated on an ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA) and visualized with GeneMapper (ver. 4.0: Applied Biosystems, Foster City, CA). MicroChecker (ver.2.2.3; Van Oosterhout *et al.* 2004) was used to evaluate genotyping errors using expected allele frequencies derived under Hardy-Weinberg Equilibrium (HWE).

Descriptive Statistics

We used Arlequin (ver 3.0; Excoffier *et al.* 2005) to calculate observed (H_o) and expected (H_e) heterozygosities for each locus and average expected heterozygosity for each cohort in the three sampling areas. We used GenePop (ver. 4.0; Rousset 2008) to calculate allelic diversity, the number of rare alleles per locus (alleles exclusive to one cohort) and deviations from HWE for each study area. HWE was examined by locus, by cohort and globally with significance determined using 10,000 dememorization steps, and 5000 iterations in each of 100 batches. We used a Bonferroni correction (single locus alpha: 0.01/10 loci=0.001) to account for multiple comparisons for ten loci during HWE tests (Rice 1989).

Genetic Comparison of Cohorts

To determine if allele frequencies differed among cohorts, contingency tests for homogeneity of allele frequencies were performed in TFGPA (ver.1.3; Miller 1997). Markov Chain Monte Carlo (MCMC) methods were used to generate a null distribution based on all possible contingency tables of allele frequencies. Then, χ^2 tests were used to determine the probability that observed allele frequencies differed among cohorts (Raymond and Rousset 1995). For each single-locus test, 5000 permutations were completed for each of 20 batches with 1000 dememorization steps. *P*-values from single-locus tests were pooled using Fisher's Combined Probability Test and multi-locus *P*-values (<0.05) were evaluated for departures from homogeneity in allele frequencies between cohorts. To prevent multi-locus tests from being dominated by a single marker, single-locus *P*-values were minimized at $P = 0.0001$ (Waples and Gaggiotti 2006). Contingency tests were performed on each cohort, and on separate male and female cohorts, with the exception of males in RAP and males and females in WI, which could not be analyzed by cohort because of insufficient samples in some birth years. Additionally, tests for differences in

allele frequencies were performed after cohorts were combined into pre-and post- populations of males, females, and all individuals (Table 4.1).

Allelic richness, or the average number of alleles per locus, was measured in pre-and post- populations (using a rarefaction index to account for differences in sample size) to determine if deer management resulted in a loss or addition of alleles from the population. Additionally, H_o, H_e , F_{IS} (inbreeding coefficient), F_{ST} and relatedness were compared to explore changes in population structure and genetic variation between pre- and post- populations. These parameters were evaluated for significant differences using a two-tailed test based on a distribution of estimates generated from 10,000 random permutations using FSTAT (ver. 2.9.3.2; Goudet 2001). In all three sampling areas, males and females were analyzed separately and combined to determine the influences of deer management on the total population and on each gender.

Tests for Reduction in Population Size

Populations that have undergone significant reductions will tend to lose low frequency alleles because of genetic drift. Thus, if low frequency classes have fewer alleles than intermediate classes (termed a mode-shift) then a population reduction (genetic bottleneck) can be inferred (Luikart *et al.* 1998). To determine if deer management resulted in a reduction in effective population size (N_e), graphical methods described by Luikart *et al.* (1998) were used to examine mode-shifts of allele frequencies between pre- and post- populations. Ten allele frequency classes were defined (0-0.1, 0.101-0.2, 0.201-0.3…0.901-1.0) per Luikart *et al.* (1998), and then the number of alleles in each class was summed for all markers. We then compared the distribution of alleles in these frequency classes in pre- and post- populations from NIL, WI, and RAP.

Since genetic drift causes alleles to be lost during a population reduction, the number of alleles will decrease, but the range of alleles will decrease only if the largest or smallest alleles are lost. Hence we expect that the ratio of the number of alleles per locus to the overall size range for the microsatellite will be smaller for populations experiencing a reduction in $N_{e.}$ This ratio, termed the M-ratio by Garza and Williamson (2001), was calculated for pre- and post-populations in NIL, WI, and RAP to examine allele frequency distributions for evidence of a reduction in N_e.

Moreover, when populations are reduced in size, the number of alleles in the population will decrease at a faster rate than the heterozygosity, thus a heterozygosity excess can indicate a genetic bottleneck or population reduction. On the other hand, a heterozygosity deficiency can indicate that the population is expanding, or receiving an influx of alleles through immigration (Cornuet and Luikart 1996). We used the program BOTTLENECK (ver. 1.2.02; Piry *et al.* 1999) to determine if heterozygosity was in excess relative to the number of alleles in the population. Following the two-phase mutation model (most mutations result in a loss or gain of a single repeat with occasion multi-repeat expansions or reductions), sign tests and Wilcoxon tests were used to determine if heterozygosity deviated from mutation-drift equilibrium (Piry *et al.* 1999). Pre- and post- populations from all three sampling areas were examined using 1000 replications of the observed data to evaluate significance ($P<0.05$).

Results

Descriptive Statistics

Multi-locus genotypes were obtained for 1,718 deer harvested in three sampling areas in the northern half of Illinois and the southern portion of Wisconsin. Global tests showed no evidence of heterozygote deficiency or excess in any of the cohorts sampled. One marker in 2004 showed

significant heterozygote deficiency, but F_{IS} was low (F_{IS} = 0.079) suggesting subpopulation structure rather than inbreeding. None of the other microsatellites violated HWE assumptions after Bonferroni corrections.

Observed heterozygosity was relatively stable across birth year cohorts, ranging from 0.69 to 0.74. These values are similar to observed heterozygosities reported for deer in Michigan (0.74; Blanchong 2003), Mississippi (0.67-0.74; DeYoung *et al.* 2003), and Kentucky (0.51-0.77; Doerner *et al.* 2005). In NIL, WI and RAP small numbers of rare alleles were detected in multiple cohorts, suggesting ongoing immigration (Table 4.1). In NIL, 77 deer (8% of samples) possessed the five rare alleles observed, with 10 of these deer being *CWD+*. In this sampling area, all rare alleles were detected in the post- population. In WI, 15 deer (3% of samples) possessed the six rare alleles observed, with three of these deer being *CWD+*. Six deer in the WI pre- population had rare alleles and nine deer in the WI post- population had rare alleles. In RAP, 21 deer (10% of samples) possessed the 10 rare alleles observed, 17 in the pre- population and four in the post- population.

Genetic Comparison of Cohorts

In general, allele frequencies were not different among most cohorts when genders were analyzed separately or combined. When males and females were analyzed together, only two contingency tests were significant; one between cohort 2002 and 2004 in RAP and one between cohort 2002 and 2005 in NIL. Allele frequencies also differed for male only cohorts in 2004 and 2005, and for female only cohorts in <1999 and 2004. However, when deer were analyzed as pre- and post- populations, significant differences in allele frequencies were observed for all deer and females alone in NIL, WI, and RAP (Table 4.2). Differences are likely attributable to

changes in female allele frequencies as males showed no significant differences between pre- and post- populations in the three sampling areas.

When genetic characteristics of pre-and post- populations were compared (Table 4.2), a significant increase in allelic richness (7.3 to 7.7; $P<0.05$) was observed in NIL, but not in WI or RAP. H_o, H_e, F_{IS}, F_{ST} and relatedness were not different for pre- and post- populations (genders combined) in any of the three sampling areas. When genders were analyzed separately, a decrease in F_{ST} (-0.003 to -0.006; $P=0.056$) and an increase in relatedness (0.001 to 0.002; $P=0.056$) was observed for post- males in NIL. Differences were not observed for males in the other two sampling areas nor for females in any of the sampling areas (Table 4.2).

Tests for Reduction in Population Size

Mode-shifts in allele frequencies for pre- and post- populations were not detected in any of the three sampling areas. In fact, the typical L-shaped distributions of allele frequencies among the ten classes were nearly identical between pre- and post- populations in NIL, WI and RAP (Fig. 4.3). M-ratios tests did not indicate population bottlenecks, though for RAP and WI, ratio values were slightly smaller for post- populations. In contrast, NIL showed a higher M-ratio in the pre-population than the post- population.

When BOTTLENECK was used to examine reductions in population size, heterozygosities did not deviate from equilibrium expectations in the NIL pre- population according to sign and Wilcoxon tests (Table 4.3). For the NIL post- population however, both sign and Wilcoxon tests indicated a heterozygosity deficiency, with the Wilcoxon test significant at $P<0.01$ and the sign test approaching statistical significance ($P=0.09$). In the WI sampling area, the pre- population showed significant heterozygosity deficiency according to Wilcoxon test ($P=0.04$), but not the sign test ($P=0.23$). For the WI post- population, the Wilcoxon tests

indicated a more significant heterozygosity deficiency ($P<0.01$), while the sign test was approaching statistical significance ($P=0.08$). In RAP the same trends were observed with no significant deviations from equilibrium in the pre- population, but significant heterozygosity deficiencies detected for both the Wilcoxon test ($P<0.01$) and the sign test ($P<0.05$) in the post- population.

Discussion

When fatal and potentially zoonotic infectious diseases like CWD emerge in wildlife species, management responses are necessary to minimize or prevent economic and ecological burdens resulting from the disease. For CWD, management maybe extremely expensive and labor intensive (Williams *et al.* 2002), and because control strategies have only recently been applied in some areas, it is difficult to determine their effectiveness in limiting disease distribution. Therefore, to ensure that management resources are being utilized efficiently, it is important to assess both epidemiological and ecological responses to management. In this study we were able to evaluate CWD management and deer population control efforts by monitoring temporal changes in genetic characteristics among cohorts using selectively neutral genetic markers. Our results demonstrate that management used in southern Wisconsin and Illinois have had an undetectable effect in males, but a measureable effect on the genetic composition of female deer in disease control areas. When populations are subjected to harvest, some genetic consequences are unavoidable (Allendorf *et al.* 2008), but the differences in pre- and post- gene diversities requires careful consideration to evaluate potential ecological impacts.

After deer management was initiated, there was an apparent influx of alleles that increased allelic richness in NIL and resulted in significant heterozygosity deficiencies in all three sampling areas. Additional alleles could have resulted from an increase in reproduction

rate, but pregnancy rates and fecundity do not show sustained increases throughout the duration of this investigation (data not shown). Alternatively, increased breeding success attributable to reduced density and mate competition could contribute additional alleles to the population. However, Clutton-Brock *et al.* (1997) reported increased breeding success in male red deer resulting from an increase in female density after the population was released from culling. We believe that immigration is the most plausible explanation for our observations, given the fact that management programs have removed a substantial number of individuals from the three sampling areas (Illinois Department of Natural Resources 2010; Wisconsin Department of Natural Resources 2010) which would have provided open niches for immigrating deer. Immigration can rapidly increase the number of low frequency alleles in a population (Cornuet and Luikart 1996). This has been documented in gray seal populations that received migrants from a distant breeding territory (Allen *et al.* 1995), and in North American gray wolves following hybridization with expanding coyote populations (Roy *et al.* 1994). Further evidence for immigration was apparent in NIL, as all rare alleles in this area were detected in the post- population, suggesting that new individuals entered the population during this time. Similarly in WI, the majority of rare alleles (9/15) were detected in the post- population. It is possible that some low frequency alleles not detectable by our sampling were lost, but if so, more were replaced through immigration.

Examining rare alleles provided evidence for temporal influx of immigrants, and also offers insight into how deer management might influence the spread of CWD. In NIL and WI, a substantial proportion of deer with rare alleles were CWD positive. In WI for instance, where CWD positive individuals comprised 7% of the total sample, 20% (3/15) of deer with rare alleles were infected with CWD. Likewise in Illinois where CWD positive deer comprised 11% of the

total sample, 13% of deer with rare alleles were infected with CWD. Since these alleles were not detected in other sampled individuals, these CWD positive deer may have been recent immigrants from another population, or near descendents of recent immigrants.

If deer management is reducing deer density, then one possibility is that immigrants from other infected locales are moving in to fill vacant niches. However, this seems unlikely since infected locales are where population reductions are greatest. One alternative is that migrant deer are more susceptible to CWD, either because of behavioral (Hölzenbein and Marchinton 1992) or genetic (Kelly *et al.* 2008) reasons, and have a higher rate of infection after migrating to a diseased locale. The implication for an uninfected herd subjected to population reduction is uncertain, though CWD influx from adjacent infected locations is a possibility. Although the number of observations is low, this finding warrants further investigation to determine the sources of immigrants and evaluate any potential risks within future management plans.

Private properties where hunting/culling is not allowed or public parks where firearms are prohibited act as refuges for deer (Hansen *et al.* 1997; Brown *et al.* 2000). Refuges can increase deer density by providing protection from harvest (Hansen *et al.* 1997). Consequently, deer occupying these ranges could be immigrating into open niches created by culling. If the genetic composition of deer residing on protected habitats differs from that of the sampled population, immigration away from these ranges could contribute to an influx of rare alleles and heterozygosity deficiencies. Of additional concern, habitat patches capable of supporting large groups of deer can exacerbate transmission of CWD by increasing contact rates among individuals, especially when the patch size is small (Wolfe *et al.* 2002; Farnsworth *et al.* 2005). Immigration away from refuges could facilitate disease movement, which is consistent with our finding of CWD positive deer with rare alleles in NIL and WI.

When populations receive immigrants, locally adapted alleles that provide a selective advantage can be overshadowed by incoming genes. This is called genetic swamping (Allendorf *et al.* 2008), and it is especially pertinent for white-tailed deer populations because polymorphisms in the gene that codes for the prion protein (*Prnp*) have been associated with resistance to CWD in Illinois and Wisconsin (Johnson *et al.* 2003; Johnson *et al.* 2006a; Kelly *et al.* 2008). Deer from the core outbreak region in Wisconsin have a higher frequency of the G96S allele, which is protective against CWD (Kelly *et al.* 2008). Thus, genetic swamping could be advantageous, as immigrants could potentially increase the frequency of G96S in the Illinois deer population. However, deer in Illinois have higher frequencies of other *Prnp* resistant alleles, and receiving immigrants could dilute these alleles or influence other locally adapted genes in the Illinois population. To determine the effects of immigration on *Prnp* genotypes, this specific gene would have to be monitored, most likely over a larger geographic and temporal scale than has been previously published, to examine changes in frequency distributions of resistant alleles.

Our observed temporal changes in allele frequencies suggest that male and female deer are differentially affected by deer management. Allele frequencies differed between pre- and post- female populations, but not in males. Similar gender-biased responses to harvest have been detected in red deer (*Cervus elaphus*), where decreases in spatial genetic structure were reported for females but not males following an increase in culling that reduced population densities drastically (Frantz *et al.* 2008). These differences in females are most likely attributable to male-biased dispersal patterns in white-tailed deer (Kelly et al. 2010). Within the study area, female dispersal is somewhat limited which results in localized genetic structuring for philopatric groups. Males, on the other hand disperse extensively, thereby homogenizing allele frequencies across the sampling areas (Kelly et al. 2010, unpublished). Therefore, males more so than

females, would be more readily able to replenish alleles lost during culling, which would result in seemingly unchanged male allele frequencies across time (Harris *et al.* 2002).

Male allele frequencies were not significantly altered by deer management. However, a slight decrease in F_{ST}, a measure of subpopulation differentiation, was observed in the post- male population indicating increased gene flow and less genetic differentiation. On the other hand, relatedness, which measures shared alleles among individuals, increased between pre- and post- populations, though the magnitude of this difference was biologically inconsequential (Queller and Goodnight 1989). Nonetheless, rare alleles detected in post- populations (Table 4.1) could have affected the calculation of identity by descent, thereby increasing male relatedness (Ritland 2000).

While examining temporal variation in genetic characteristics has provided insight into the consequences of deer management, this investigation is not without limitations. Some areas in NIL and WI were not consistently sampled each year and had greater sample numbers in younger cohorts, and this may have allowed us to detect additional rare alleles in later sampling years. The same geographic areas were consistently sampled in RAP, but only 70% of the TRS in WI and 51% of the TRS in Illinois were sampled before and after the initiation of CWD deer management programs. Therefore, some of the rare alleles in the post- population may have been detected because additional TRS were sampled and not because of immigration. However, corrections for sample size differences (such as those used during the calculation of allelic richness) were employed to account for variability in sampling when pre and post- populations were compared. Further, tests for heterozygosity deficiencies would be unaffected by inconsistent sampling because pre- and post- estimates of heterozygosity are calculated independently, considering only the distributions of alleles within the sampled population.

Overall, genetic monitoring has proven extremely useful for examining genetic consequences of deer management in Illinois and southern Wisconsin. Contrary to several studies that reported losses of genetic diversity in cervid populations after harvest (McCullough *et al.* 1996; Martinez *et al.* 2002; Nabata *et al.* 2004), selectively neutral genetic markers revealed that the number of alleles in managed populations is increasing, most likely because of immigration. Deer removal provides unoccupied niches for immigrants, which could result in an influx of CWD resistant *Prnp* alleles into northern Illinois and southern Wisconsin. However, unoccupied habitats could also attract CWD infected deer from the disease core in Wisconsin. Further, given the potential for environmental transmission of CWD, population turnovers could inadvertently expose uninfected immigrants to infectious prions, and these risks need to be considered when management plans are implemented. Though some of the disadvantages associated with culling-induced immigration seem serious, they pale in comparison to the risk of CWD spread in an unmanaged population (Gross and Miller 2001). The economic burden of managing CWD would only elevate if additional areas became infected, therefore, as long as culling continues to reduce CWD prevalence (Weng et al. 2010, in preparation), it should be continued but with additional monitoring to ensure that genetic consequences do not become too severe.

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Table 4.1. Sample size (*n*), observed heterozygosity, and number of alleles (both common and rare to one cohort) detected in deer from NIL, WI, and RAP.

Study Site	Cohort	*n*	Temporal Population	Observed Heterozygosity*	Number of Alleles	Number of Rare Alleles
NIL	<1999[1]	36	Pre-cull	0.73	75	0
	2000	31		0.71	81	0
	2001	85		0.72	94	0
	2002	145		0.72	92	0
	2003	137	Post-cull	0.72	105	1
	2004	145		0.70	109	1
	2005	318		0.72	112	3
	>2006[2]	23		0.69	80	0
WI	<1999	0	Pre-hunter harvest cull	na	na	na
	2000	2		0.69	76	3
	2001	18				
	2002	250		0.71	111	1
	2003	13	Post-hunter harvest cull	0.74	87	1
	2004	14				
	2005	182		0.71	97	1
	>2006	0		na	na	na
RAP	<1999	0	Pre-cull	na	na	na
	2000	0		na	na	na
	2001	33		0.74	83	2
	2002	57		0.72	88	1
	2003	49		0.71	92	2
	2004	55		0.71	96	2
	2005	61	Post-population control cull	0.71	99	3
	>2006	22		0.77	79	0

*Averaged across 10 loci. Rows highlighted in grey had low *n* and were combined into a single cohort for analysis. [1] Includes deer born 1998-1999. [2]Includes deer born 2006-2008. "na" indicates that the cohort was not sampled.

Table 4.2. Genetic differences between pre- and post- populations of white-tailed deer.

Study Area	Total Sample	Males	Females
IL	Allele Frequencies*** Increase Allelic Richness*	Decrease F_{ST}* Increase Relatedness*	Allele Frequencies**
WI	Allele Frequencies***	None	Allele Frequencies**
RAP	Allele Frequencies*	None	Allele Frequencies*

* P <0.05; ** P <0.001; *** P <0.0001;

Table 4.3. *P*-values for heterozygosity equilibrium tests conducted on pre- and post- populations of white-tailed deer in NIL, WI, and RAP.

	Pre- Population		Post- Population	
Sampling Area	Sign Test[1]	Wilcoxon Test	Sign Test	Wilcoxon Test
NIL	0.22	0.14	0.09	0.02
WI	0.23	0.04	0.08	0.01
RAP	0.55	0.28	0.02	0.01

[1] All reported tests indicated heterozygosity deficiency.

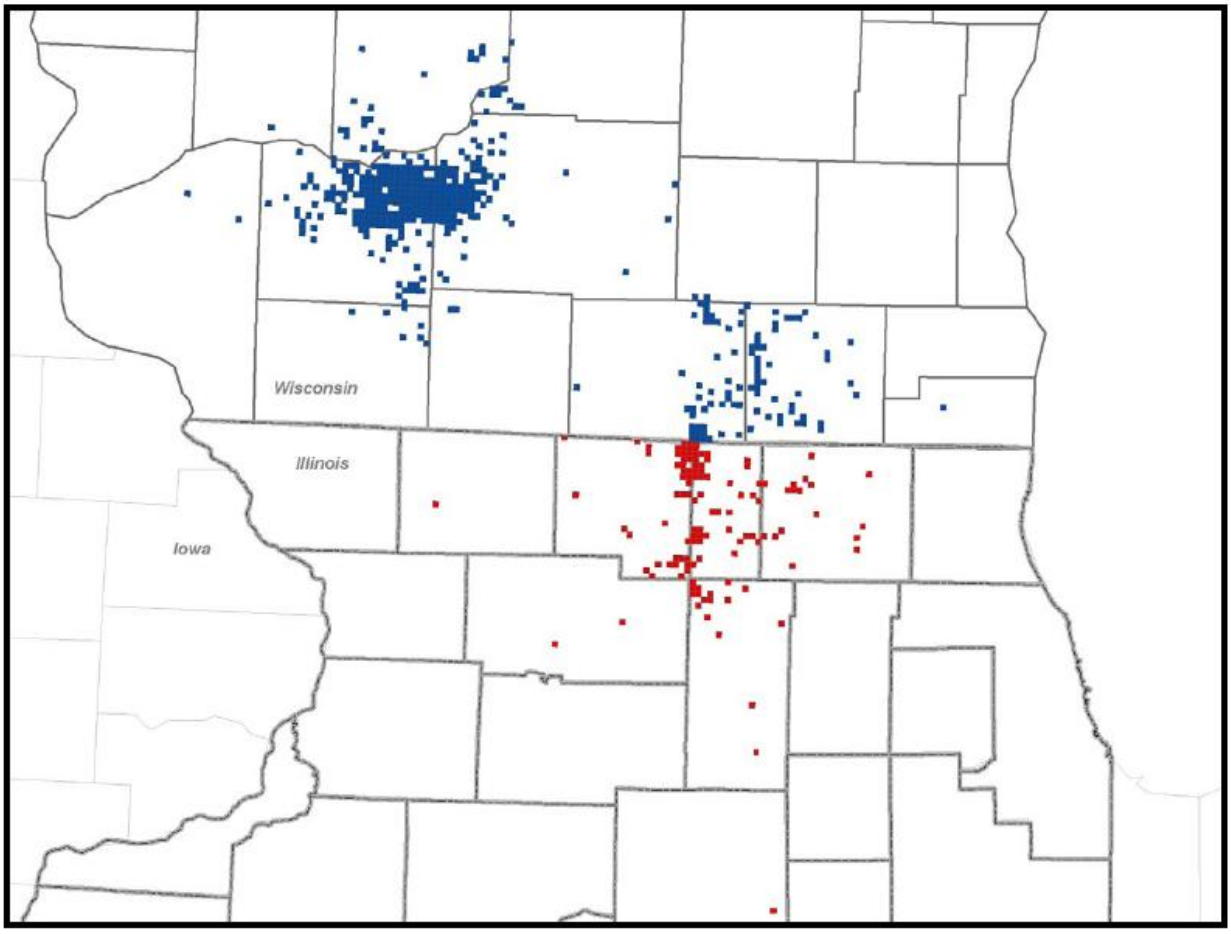

Figure 4.1. Distribution of CWD in southern Wisconsin and northern Illinois as of June 30, 2009. Squares represent sections in which CWD has been detected. Map courtesy of Illinois Department of Natural Resources.

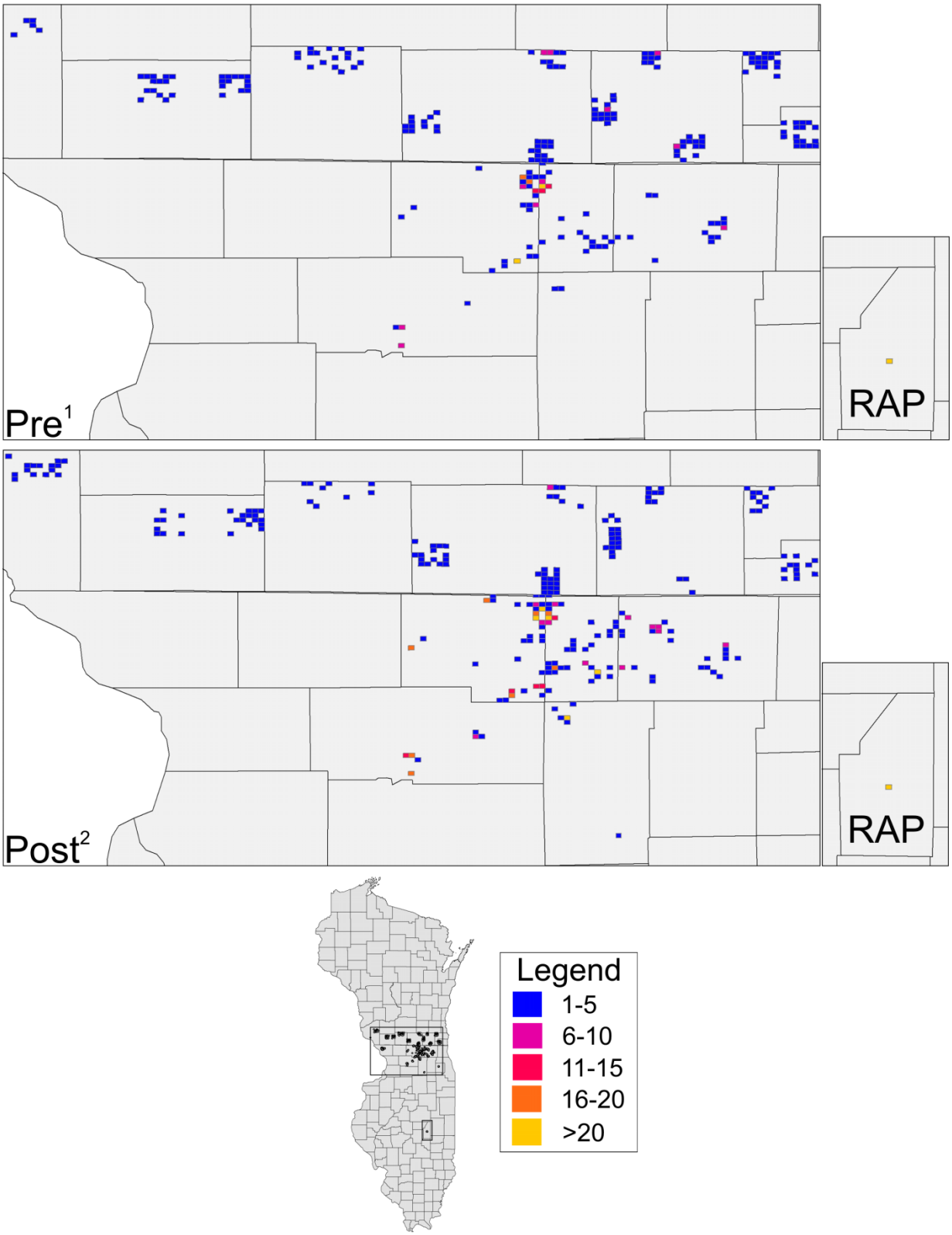

Fig. 4.2. Distribution and sampling intensity of deer in pre- (top panel) and post- (bottom panel) populations in IL, WI, and RAP (inset). [1] Includes deer born 1998-2002. [2]Includes deer born 2003-2008.

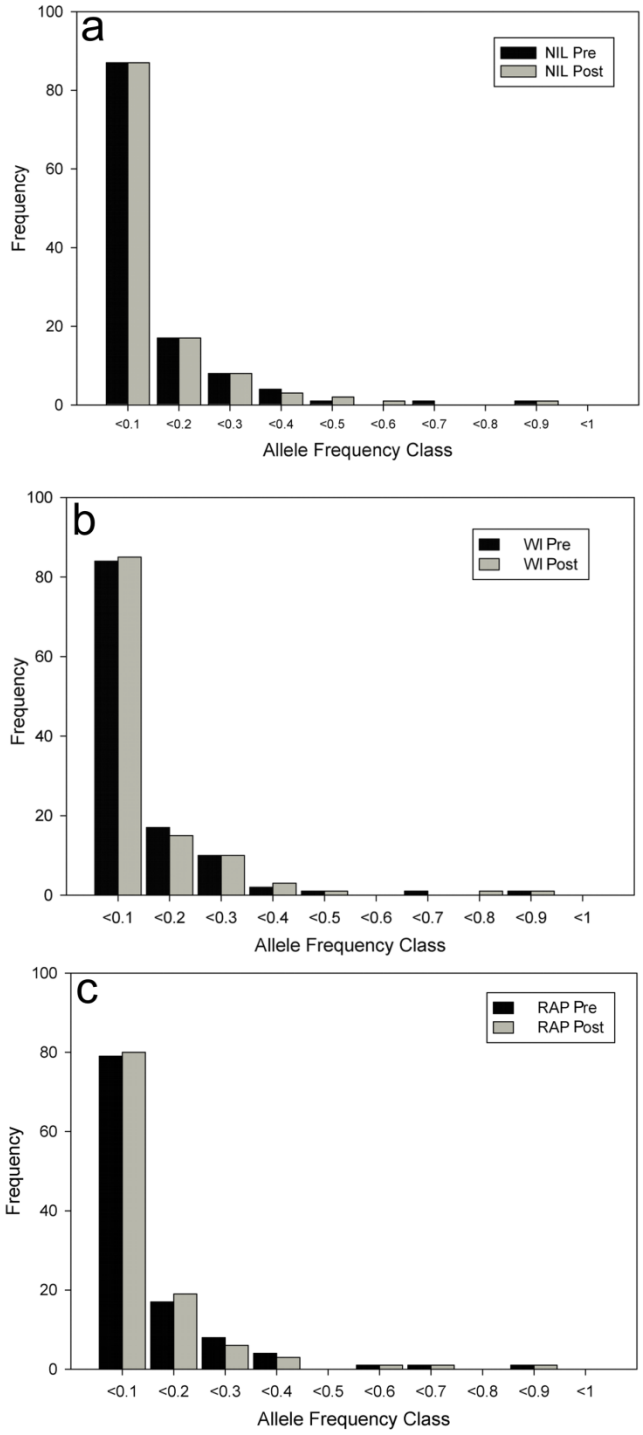

Figure 4.3. Allele frequency distributions for pre- and post- populations in NIL (panel a), WI (panel b), and RAP (panel c). Allele frequencies were determined for deer born in pre- and post-populations. Ten allele frequency classes were defined and examined for mode shifts.

Chapter 5: Landscape genetics of white-tailed deer inhabiting the CWD outbreak region of Illinois and southern Wisconsin.

Abstract

In this investigation, we used landscape genetics to examine the effect of landscape features on dispersal and population boundaries of white-tailed deer occupying the chronic wasting disease (CWD) infected region of Illinois and southern Wisconsin. An awareness of how the landscape affects animal movement and genetic exchange between populations contributes to our understanding of wildlife ecology. This knowledge has become particularly important in the Midwest since CWD was discovered there in 2002. Because CWD is an infectious prion disease, deer management to prevent or reduce transmission and spread is crucial. By quantifying genetic structure across the landscape we have identified populations with high and low admixture and discovered gender specific barriers to deer movement that may contribute to CWD spread via dispersal. We found that rivers, streams and interstates contributed to the genetic structuring of females in the study area, but males were insensitive to these features. The observed variations in landscape use between males and females implies that CWD could spread via male movement relatively independently of natural and manmade landscape features, while CWD spread by females would occur over shorter distances because movement is inhibited by these landscape features. This is the first deer landscape genetics study to evaluate influences of environmental barriers across the diverse habitats of the Midwestern US, and it contributes novel information relevant to the management of white-tailed deer and infectious diseases in wildlife populations.

Introduction

Landscape features such as forests and streams determine the distribution and abundance of wildlife species because they provide food, water and shelter for local fauna (Andrewartha and Birch 1986). Similarly, environmental features such as rivers or mountains can influence dispersal within and among populations by altering accessibility to resources (Holderegger and Wagner 2008). Landscape use varies among wildlife species because each has specific resource requirements and habitat preferences. Human perceptions of "suitable habitat" or "barriers to movement" are not always consistent with the species perception of the environment (Wiens 2001). Thus, investigating how the landscape affects animal movement, abundance, and the distribution of populations is essential for understanding wildlife ecology.

With recent advancements in genetic technology, relationships between animals and their environment can now be explored using genetic data. Landscape genetics is an emerging field that uses genetic tools to determine how the environment influences genetic exchange among populations (Manel *et al.* 2003). Natural landscape features such as rivers, forests, and mountains, or man-made features such as roads and cities may influence characteristics such as gene variability within a population or the range of genetically similar populations. Landscape genetics can guide wildlife management and conservation by clarifying environmental factors important to animal distribution and variation. In general, genetic variation contributes to healthy populations and is a consideration in wildlife conservation and management, while genetic differentiation indicates population ranges and population structure which are of additional interest to managers. Landscape genetics is also useful for addressing contemporary ecological issues such as invasive species (Zalewski *et al.* 2009), habitat fragmentation and human land use

conflicts (Vandergast *et al.* 2007), because it is applicable in a variety of species and habitat types.

Landscape genetics provides information on wildlife dispersal and movement that cannot be easily obtained from direct observation of a species by telemetry, mark recapture, or similar techniques (Holderegger and Wagner 2008). In bighorn sheep for example, landscape genetics revealed that interstates and canals were barriers to movement, and the sheep were at increased risk for metapopulation extinction by decreased genetic diversity. Based on landscape genetics, the authors suggested constructing over- and underpasses to promote connectivity and preserve the genetic viability of big horn sheep in California (Epps *et al.* 2005). In Scotland, Zalewskit *et al.* (2009) found that mountain ranges were incomplete barriers to invasive mink, and the authors suggested eradication efforts focus on genetic admixture zones along mountain ranges to prevent further invasion.

Several studies in cervids have recently demonstrated the value of landscape genetics in the context of game species management. Frantz et al. (2006) demonstrated illegal translocation of red deer by identifying individual deer that were genetically distinct and belonged to a geographically distant population. Coulon *et al.* (2006) were able to define "ecologically meaningful management units" for roe deer based on genetic populations bounded by landscape barriers. Previously, roe deer management units in France were based on administrative boundaries, and their redefinition based on landscape genetics was a more ecologically meaningful approach to population management (Coulon *et al.* 2006). In this investigation, we applied landscape genetics to characterize barriers to dispersal and delineate populations of white-tailed deer in the Midwestern United States. White-tailed deer are abundant in this area and managers currently rely on public hunting and controlled herd reductions to regulate

populations (Illinois Department of Natural Resources 2010; Wisconsin Department of Natural Resources 2010). Consequently, information about population processes such as dispersal that contribute to genetic variation would be valuable and could be incorporated into the design and implementation of management plans.

White-tailed deer in the Midwest occupy a continuum of habitats that range from fragmented agricultural landscapes in Illinois (Nixon 1991) to closed canopy forests in Wisconsin and Michigan (Scribner *et al.* 2005). In this region, fragmented forests have been associated with increased deer dispersal (Nixon *et al.* 2007). Similarly, open canopies as indicated by thermal cover potential promote movement among populations (Scribner *et al.* 2005). Elevated dispersal can result in low genetic differentiation across large geographic areas in fragmented habitats (Kelly *et al.* 2010, submitted), however certain landscape features have been reported to deter deer movement. Blanchong *et al.* (2008) found that the Wisconsin River was a barrier to deer movement and Kelly *et al.* (2010) identified significant differences in allele frequencies for Illinois deer populations separated by an interstate highway (hereafter, "interstate").

Understanding landscape use in white-tailed deer has become particularly important in the Midwest since chronic wasting disease (CWD), was discovered in the Midwest in 2002. Because CWD is an infectious and fatal prion disease, deer management has become imperative to preventing further transmission and spread (Weng et al. 2010, in preparation). By identifying barriers to deer movement and quantifying genetic structure across the landscape, we can better define dispersal patterns that may contribute to CWD spread and transmission.

We used ten microsatellite markers to examine gene flow and define populations of white-tailed deer occupying the CWD infected region of Illinois and southern Wisconsin. Our

main objective was to evaluate whether rivers, streams, interstates and highways were barriers to gene flow, and thereby contributed to genetic structuring of these populations. Though Blanchong *et al.* (2008) applied landscape genetics in populations in southwestern Wisconsin, and Kelly *et al.* (2010) examined population genetics of deer in northern Illinois, this is the first landscape genetics study to evaluate influences of environmental barriers at a regional level that encompasses a highly varied landscape.

Materials and Methods

Deer Sampling Areas

We utilized a subsample of deer tissue samples collected through CWD surveillance and population control programs targeting northern Illinois and southern Wisconsin. The majority of samples were collected from areas at increased risk for disease based on proximity to prior CWD cases. To expand the geographic range of our sampling, we also obtained samples collected through deer population reduction programs conducted in areas ~300 km from northern Illinois and southern Wisconsin. Harvest date, gender, age and spatial locations were collected for all samples. Because deer were harvested for disease surveillance and population control and not for genetic analysis, we were not able to conduct an experimental study with a hypothesis driven design. Rather, we conducted a genetic survey on tissues that were available through disease control efforts.

North-central Illinois sampling area (NIL). Between January 2003 and March 2008, Illinois Department of Natural Resources (IDNR) harvested ~5,000 free-ranging deer in the CWD-infected region of northern Illinois (Fig. 5.1). Of the samples collected, 921 from Winnebago, Boone, DeKalb, Ogle, and McHenry counties were used for genetic analysis. For deer sampled in NIL, spatial locations were recorded to the nearest township/range/section (TRS; 2.6 km^2).

North-western Illinois sampling area (GTA). Between January and February 2008, 219 free-ranging deer were harvested in population control programs from Galena Territory Association in JoDaviess County (Fig. 5.1). For deer sampled in GTA, spatial locations were recorded to the nearest 0.1 km.

North-eastern Illinois sampling area (DuP). Between December 2007 and January 2008, 50 free-ranging deer were harvested by United States Department of Agriculture Wildlife Services personnel through deer management programs initiated by the DuPage County Forest Preserve (Fig. 5.1). For deer sampled in DuP, spatial locations were recorded to the nearest township/range/section (TRS).

East-central Illinois sampling area (RAP). During fall hunting seasons between 2005 and 2007, 319 free-ranging deer were harvested at University of Illinois Robert Allerton Park (RAP) through their Deer Management and Research Program (Fig. 5.1). For deer sampled in RAP, spatial locations were recorded to the nearest km.

Wisconsin sampling Area (WI). Between October 2003 and March 2006, Wisconsin Department of Natural Resources (WDNR) collected samples from 71,611 free-ranging deer harvested by hunters in the CWD management zone in southern Wisconsin. Of the samples collected, 479 from Grant, Lafayette, Green, Rock, Walworth, Racine, and Kenosha counties were used for genetic analysis (Fig. 5.1). For deer sampled in WI, spatial locations were recorded to the nearest township/range/section (TRS).

Laboratory Procedures

Muscle samples were stored in 100% ethanol and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison WI). Individuals were genotyped using 10 microsatellite DNA primers previously described in Kelly et al. (2010). Forward primers were

labeled with fluorescent dyes (NED, HEX, FAM) with fragments separated on an ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA) and visualized with GeneMapper (v. 4.0: Applied Biosystems, Foster City, CA). MicroChecker (v.2.2.3; Van Oosterhout *et al.* 2004) was used to evaluate genotyping errors using expected allele frequencies derived under Hardy-Weinberg Equilibrium (HWE). We also used GenePop (ver. 4.0; Rousset 2008) to examine deviations from Hardy Weinburg Equilibrium (HWE) by locus, by sampling area and overall with significance determined using 10,000 dememorization steps, and 5000 iterations in each of 100 batches.

Landscape Features

The distribution of genotyped deer in the four sampling areas was visualized using ArcGIS (ver. 9.2; ESRI 2006). ArcGIS was also used to map US roads as defined by the North American Atlas data (downloadable from nationalatlas.gov), rivers and streams as defined by the National Hydrography Dataset, (downloadable from the U.S. Geological Survey, http://www.usgs.gov). We chose to evaluate interstates, highways, rivers and streams based on previous genetic analyses implicating these types of landscape features as barriers to white-tailed deer gene flow (Blanchong *et al.* 2008); Kelly *et al.* 2010, submitted). Deer were allocated into 31 study sites based on geographic proximity of samples and their geographic distribution in relation to landscape features of interest (Fig 1). For example, deer on opposite sides of an interstate or river were allocated into separate study sites so that the influence of the landscape on genetic exchange could be quantified. Figure 2 shows the number of deer (*n*) in each study site. Three binary distance matrices were created for landscape features; one for interstates, one for highways and one for rivers/streams. In these matrices, a value of one indicated that the landscape feature was present between study sites, whereas a value of zero indicated no barrier.

Additionally, pairwise geographic analog distances calculated from the mean center of each study site (weighted by sample number) were compiled into a matrix so that we could evaluate the effects of geographic distance on gene flow.

Evaluating Barriers

Because the probability of encountering a landscape feature tends to increase with distance, to an extent, we would expect that binary landscape matrices would be correlated with geographic distance matrices. To account for these correlations, we used partial Mantel tests (Smouse *et al.* 1986) to separate out the predictive value of each distance matrix and determine which acted as barriers to gene flow. Partial Mantel tests perform linear matrix regression analysis using multiple distance matrices (X: geographic distance, barrier distance) to predict a response variable (Y: F_{ST}). These analyses are able to account for redundancies among X matrices so that the additional explanatory power offered by the inclusion of each predictor variable can be assessed (Smouse *et al.* 1986). For this analysis, a genetic distance matrix of F_{ST} values between the 31 study sites was generated in Arlequin (ver. 3.1; Excoffier *et al.* 2005) for males, females, and all deer combined. We then performed separate partial Mantel tests on each of the three binary landscape distance matrices with F_{ST} as the dependent variable and geographic distance as a covariate. Statistical significance of each correlation was determined through 10,000 random permutations of the observed data. Landscape variables were evaluated for males and females separately and also for both genders combined.

Effects of Barriers on Population Structure

To examine the congruence of genetic population boundaries with landscape features, we inferred population structure using a Bayesian method to assign individuals to genetically similar clusters. Bayesian clustering was implemented in STRUCTURE (v. 2.3.1; Pritchard *et al.* 2000)

which estimates the natural logarithm of the probability [LnP(D)] that individual genotypes belong to a given cluster (*k*=number of clusters to which individuals are assigned during analysis). Assignment of individuals eliminates the need for *a priori* population definitions. Twenty replicates were run for *k* values 1 through 7 and the simulation yielding the smallest Bayesian deviance was selected as the optimal model for the number of clusters (Pritchard *et al.* 2000; Faubet *et al.* 2007).

The Bayesian method implemented in STRUCTURE can incorporate background information about the population to better examine admixture. An admixture model (initial α =1.0, max α = 10.0, SD of α = 0.05) was computed with TRS as the location for each deer and correlated allele frequencies specified to account for shared ancestry (Pritchard *et al.* 2000; Falush *et al.* 2003). To facilitate interpretation of results, study sites were specified as the population identifiers for STRUCTURE, so their respective F_{ST} values could be compared to simulated admixture proportions. Initially, several pilot simulations were run to determine burn-in length and consistency of parameter estimates across replicates. Clusters were simulated using a burn in of 100,000 MCMC steps followed by 100,000 replicates to estimate posterior probabilities of all parameters. Individuals were assigned to the inferred cluster containing the highest percentage of membership (q).

Results

Multi-locus genotypes from deer in all five sampling areas followed HWE when microsatellites were tested globally. However, one marker (RT23) in NIL showed significant heterozygote deficiency, but F_{IS} was low (F_{IS} = 0.018) suggesting subpopulation structure rather than inbreeding. The number of alleles per locus observed within a sampling area (average = 11) ranged from 2 (OarFcb304) to 19 (RT23).

Evaluating Barriers

Partial Mantel tests revealed that rivers/streams were barriers to movement in females, but not males. The presence or absence of rivers/streams between populations was significantly (P<0.05) correlated with pairwise genetic distances (F_{ST}) between study sites for females, and accounted for additional variance in F_{ST} values (Table 5.1) beyond that explained by geographic distance. Interstates also appeared to influence female movement though the added explanatory power was small, and the correlation approached, but did not reach, statistical significance (P=0.08). Consistent with previous studies of deer movement in Wisconsin (Blanchong *et al.* 2008), highways were not significantly correlated with genetic distances in females. In contrast, none of the three landscape distance matrices were associated with genetic distance in males suggesting that these environmental features do not impede male movement. However, when all deer were analyzed together, rivers and streams were significantly correlated with genetic distance and they explained an additional 2.24% of the variation in F_{ST} values.

Effects of Barriers on Population Structure

A five cluster model produced the lowest Bayesian deviance (Fig. 5.2). Although all five were in HWE (Bonferroni corrected P<0.001), cluster membership for some study sites was divided across multiple clusters. A simulation with four clusters produced a similarly low Bayesian Deviance value and also divided membership at some study sites. Nonetheless, the five cluster model placed GTA and RAP into separate clusters and divided NIL and WI into three clusters; one spanning the southern border of Wisconsin, one to the northeast of Rockford, and one to the southwest of Rockford. The RAP study site was the most genetically distinct (at 90% membership). Deer from GTA were less distinct than RAP but more distinct than the other clusters with 66% of membership assigned to their respective cluster. For study sites in WI,

NILNE and NILSW, admixture was high along contact zones, and for a few study sites, membership was more or less evenly divided between adjacent clusters, indicating extensive gene flow along the exterior of the inferred populations (Fig. 5.2).

Discussion

Our evaluation of landscape features in Illinois and southern Wisconsin revealed that genetic structure in white-tailed deer was shaped by rivers and streams more so than roads, though interstates did contribute to population delineation in some instances. Our findings are consistent with another landscape genetics study of deer in the Midwest where US Highway 18 in Wisconsin did not deter gene flow, but the Wisconsin River was found to be a barrier to deer movement (Blanchong *et al.* 2008). Interestingly, white-tailed deer are known to be good swimmers and often cross large streams when avoiding predators (Halls 1984), so our findings are somewhat unexpected. However, predation is not the leading cause of mortality for deer in the study area (Nixon 1991), and consequently, this behavior may not be common for the sampled population. Moreover, small tributaries and streams were not included in the analysis, and the vast majority of rivers and streams examined were > 80 feet wide which could deter many deer from swimming across. An evaluation of smaller streams, or the inclusion of variables such as water depth and current speed would be of interest to further define characteristics of streams and rivers that deter deer crossing.

The gene flow barriers detected with partial Mantel tests were clearly not absolute barriers to movement. Correlations were weak and study sites on opposite sides of interstates and rivers were assigned to the same cluster in some cases. It is possible that interstates represent permeable barriers because deer can use underpasses to cross interstates and highways, which has been widely documented in Illinois and several other states (Ward 1982; Foster and

Humphrey 1995; Etter *et al.* 2002; Ng *et al.* 2004). In fact, underpasses have been constructed in some locations as a way to reduce deer-vehicle collisions because these structures create movement corridors for deer (Mastro *et al.* 2008). Besides underpasses, certain habitat features may encourage deer to cross roads. Finder *et al.* (1999) found that deer vehicle collisions in Illinois were higher when roads were close to forests and parks, or intersected a riparian corridor. These landscape features were related to preferred habitat and deer density, which implies that deer were crossing roads near these features to gain access to cover or food (Waring *et al.* 1991; Finder *et al.* 1999).

There were four sites in the study area where interstate overpasses crossed large riparian corridors perpendicularly requiring a sizeable overpass (Fig. 5.2; I-39 between sites 18 and 21, I-90 between sites 21 and 22, I-90 between sites 5 and 6, I-90 between sites 29 and 30). In two of these locations, study sites on opposite sides of the interstate were assigned to the same cluster. In addition to the habitat surrounding the road, the number of lanes, traffic volume and the absence of fences have been shown to increase deer vehicle collisions in other areas (Gunson *et al.* 2005), and while these variables were not included in this investigation, evaluating them in terms of gene flow and population structure would help managers assess the risk for future collisions in our region.

Our results suggest that male and female deer use the landscape differently. Rivers, stream and interstates were significantly correlated to female, but not male F_{ST}, indicating that these features are less important in determining male movement. In deer, different genders have specific biological roles, and for females, behaviors tend to revolve around fawn rearing (Halls 1984). Observational studies have shown that does are cautious and alert when crossing roads, but fawns are inexperienced and tend to be oblivious to possible hazards (Waring *et al.* 1991), so

females may perceive interstates as barriers to protect their new fawns. Similarly, young fawns are not very mobile (Nixon 1991; Nelson and Woolf 1987) and they may be physically unable to swim across a stream or river, which could deter their mother as well. In contrast to females, males tend to be the dispersing sex in deer (Halls 1984). Inbreeding avoidance (Hoelzenbein and Marchinton 1992) or resource competition (Nixon 1991) can drive extensive male movement (Long *et al.* 2008), perhaps without regard to landscape features. In Pennsylvania, observation indicated males crossed interstates more frequently than females (Feldhamer *et al.* 1986) and in Wisconsin, more males were killed by vehicle collisions than females despite a sex ratio that was biased towards does in the population (Jahn 1959). These findings, in conjunction with our own genetic observations, suggest that environmental perceptions vary between genders. These features influence movement across the landscape differently in male and female deer.

The objective of this study was to determine whether some landscape features were barriers to deer movement, though in doing so we have ignored landscape features that may be conducive to gene flow. Considering that forest cover has been related to movement in deer (Blanchong 2003; Long *et al.* 2005) and other large mammals (Cushman *et al.* 2006), the exclusion of this variable from our analysis prevents us from looking at effects of deer habitat on population structure and movement. We did not included forest cover because this variable can be difficult to measure, and methods to quantify vegetational fauna were not available at the time of analysis. Additionally, two of the inferred clusters (NILNE and NILSW) were separated by an interstate, a river, and a large city (Rockford), and so inferences about barriers in this area are confounded because urbanization was not evaluated. We are further limited by our choice of study site as the unit of analysis, as assessing individuals would have allowed us to maximize the information that went into the regression model (Cushman *et al.* 2006). However, the study sites

used represent a high percentage of locations where samples could be legally obtained, and in some areas study sites represent deer habitable fragments in a non-continuous landscape. Use of individual-level data was limited by available software and the large sample size. Future investigations will need to focus on quantifying the effects of forest, agriculture, riparian corridors and urbanization on the movement of individuals.

Despite these limitations, we were able to evaluate the effects of some landscape features on the movement of deer populations distributed across two states, a task that would have been challenging using direct measures of movement. Our results suggested that rivers, streams, and interstates were predictors of genetic structure, although males appear insensitive to these barriers compared to females. For wildlife managers these results are important because the lack of gene flow barriers for males implies extensive movement and suggests that CWD could spread via male dispersers regardless of natural and manmade landscape features. CWD spread mediated by females, on the other hand, would be slowed by large rivers and streams, and to an extent interstates, but in either case these barriers would not cease movement or eliminate the risk of disease spread across the landscape. This study contributes to the management of white-tailed deer in CWD infected regions and demonstrates the utility of landscape genetics in describing animal movement and clarifying landscape use. In the future we plan to develop methodologies that will allow us to evaluate additional landscape variables and incorporate individual level genetic data so that we can further guide deer managers.

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Table 5.1. Correlation coefficients (r), significance (*P*), and the percent of additional variance in F_{ST} explained (Added σ^2) by environmental variables in white-tailed deer.

Landscape Variable[1]	All Deer			Females			Males		
	r	*P*	Added σ^{2*}	r	*P*	Added σ^{2*}	r	*P*	Added σ^{2*}
Interstates	0.08	0.12	ns	0.10	0.08	0.87%	0.03	0.35	ns
Highways	0.05	0.23	ns	0.02	0.35	ns	0.06	0.20	ns
RiversandStreams	0.17	0.01	2.24%	0.18	0.01	3.35%	0.03	0.38	ns

*Additional variance explained after correlations between F_{ST} and geographic distance and correlations between geographic distance and landscape variable have been accounted for.
ns: Correlations were not significant ($P<0.1$).
[1]Analysed as a binary value, present or absent between pairs of study sites

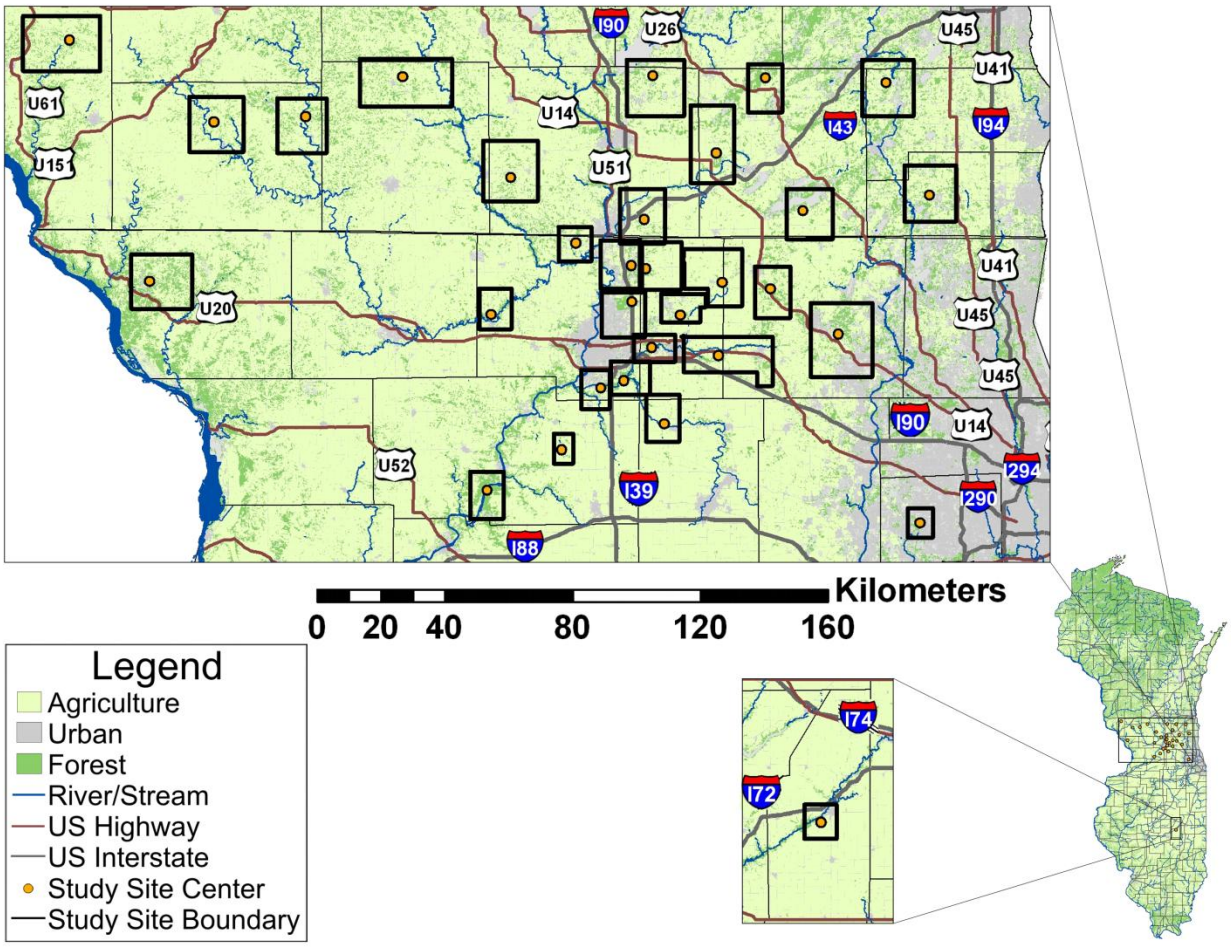

Figure 5.1. Map of the study area in southern Wisconsin and Illinois. Deer were allocated into 31 study sites on geographic proximity of samples and their geographic distribution in relation to landscape features of interest. Black boundaries surround the distribution of samples around each study site's mean center (orange dots). If straight lines connecting mean centers of two study sites intersected an interstate, highway or river/stream, a value of one was entered into the landscape feature matrix, if not the value was entered as zero.

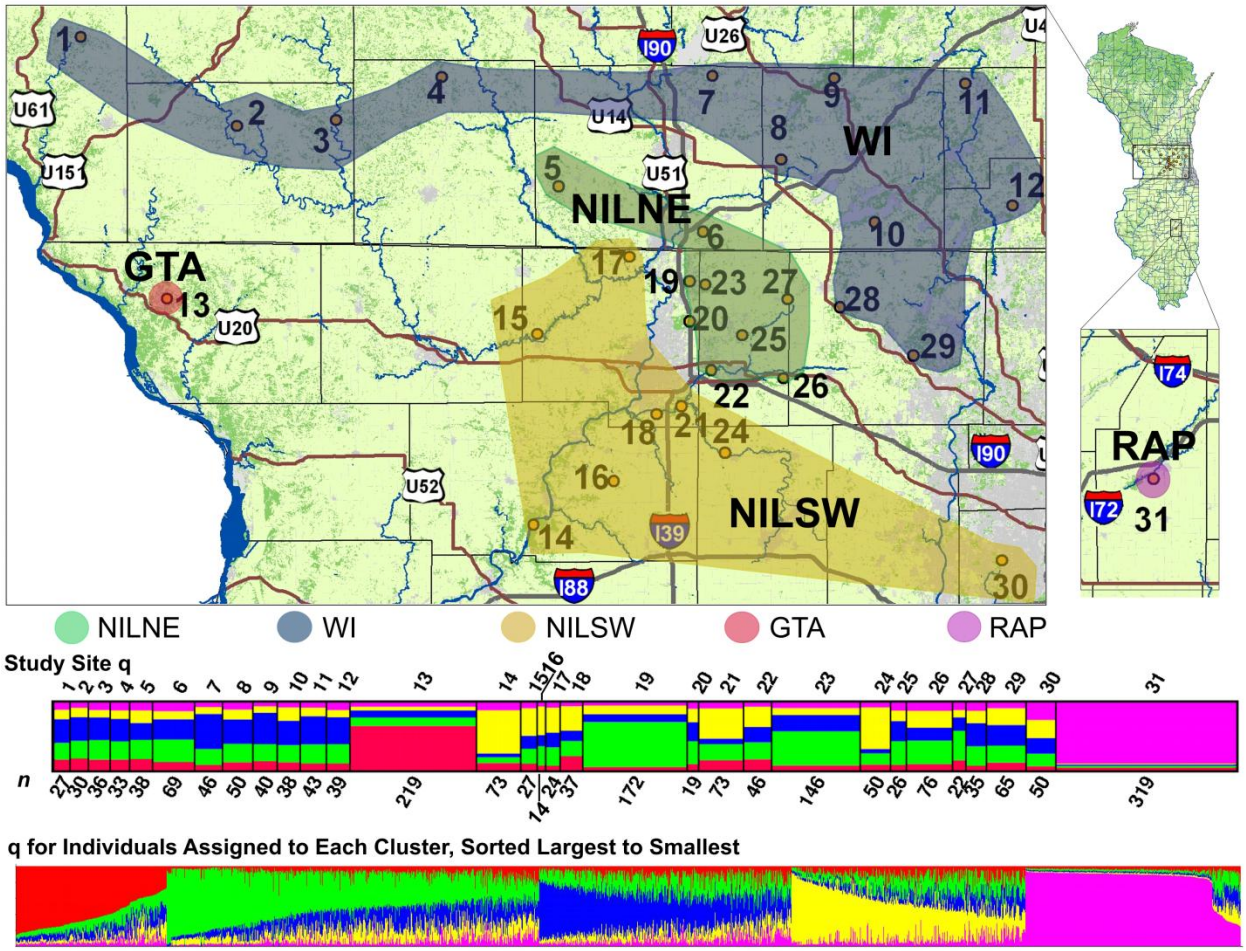

Figure 5.2. Genetic populations of deer in Illinois as revealed by Bayesian assignment of individuals. Clusters were simulated using STRUCTURE and study sites were assigned to the inferred cluster which contained the highest percentage of membership. Green represents membership assigned to NILNE, yellow represents membership assigned to NILSW, red represents membership assigned to GTA, blue represents membership assigned to WI, and pink represents membership assigned to RAP. Membership proportions (q) in the five inferred clusters are shown for all 31 study sites (top bar chart, sites separated by black vertical lines) with *n* for each study site displayed along the bottom. Individual q values (bottom bar chart) were sorted from high to low for each inferred cluster to demonstrate admixture among adjacent populations.

Chapter 6: Beyond State Borders: Landscape Genetics and CWD in White-tailed deer from Illinois and southern Wisconsin

Abstract

In this study we used molecular techniques to examine deer movement and population structure in the context of chronic wasting disease transmission and spread. Chronic wasting disease is an infectious prion encephalopathy in cervids that is endemic to Colorado and Wyoming but has spread across the US within the last decade. Quantifying white-tailed deer movement and population structure in infected areas can facilitate predictions of CWD spread via deer dispersal. We analyzed microsatellite genotypes of white-tailed populations in southern Wisconsin and Illinois to quantify population level movements, admixture and gender-biased dispersal patterns using F_{ST} and contingency tests. We also examined movements of individuals using assignment tests and spatial autocorrelation, and quantified dispersal events using parentage assignment. Finally, we compared genetic characteristics such as allelic diversity, heterozygosity and fixation indices between CWD infected and uninfected individuals to determine if CWD affects movement of white-tailed deer. Genetic characteristics were not different between CWD infected and uninfected deer, suggesting that changes in movement behaviors associated with clinical illness were not detectable with our molecular data. We found that both male and female deer move extensively in northern Illinois and southern Wisconsin, and that this movement could facilitate CWD spread via dispersal. In contrast, a few locations demonstrated reduced deer movement and female philopatry. One of these locations is a hotspot for CWD in Illinois, and it appears that reduced movements in this area could be exacerbating CWD transmission via direct contact among deer. The observed spatial heterogeneity in deer

movement and population structure has important management implications as it allowed us to identify locations at risk for future CWD infection and areas in need of management.

Introduction

Infectious pathogens and their hosts are influenced by the environment, which in turn, affects disease transmission, spread, and persistence. Physical attributes of the landscape such as mountains (Zalewski *et al.* 2009) or rivers can impede dispersal, thereby limiting host distribution and the spatial extent of infection (Cullingham *et al.* 2009). However, landscape features can modify disease transmission and prevalence by supporting large host density in isolated habitats (Hess 1996; Farnsworth *et al.* 2005). Furthermore, forests and riparian corridors can promote host movement and facilitate pathogen spread via dispersal (Real and Biek 2007).

Determining how the landscape mediates host movement and the distribution of populations can provide insight into disease dynamics. With this information, wildlife managers can identify areas at risk for pathogen influx that should be targeted for disease surveillance. Alternatively, managers may choose to reduce control efforts in areas experiencing reduced host movement and disease spread attributable to a landscape barrier (Cullingham *et al.* 2009).

Chronic wasting disease (CWD) is an infectious prion encephalopathy in cervids that is endemic to Colorado and Wyoming but has spread across the US within the last decade. The environment affects disease dynamics in several ways. First, infectious prions deposited in the environment through feces or carcasses can transmit disease (Miller *et al.* 2004; Miller *et al.* 2006). Further, soil particles like clay bind to infectious prions with higher affinity than sand (Johnson *et al.* 2006), therefore, soil composition could influence CWD persistence in the environment.

Additionally, landscape features influence the movement and distribution of cervids which in turn affects CWD dynamics. Human development can concentrate deer in small habitats (Wolfe *et al.* 2002) and lead to higher CWD prevalence in developed rather than undeveloped areas (Farnsworth *et al.* 2005). Moreover, CWD appears to spread via cervid dispersal (Conner and Miller 2004), and landscape features like rivers have been implicated as barriers to deer movement and disease spread (Blanchong *et al.* 2008).

Currently, CWD in some states is being managed by culling to decrease deer density, disease transmission and the number of infected individuals from the population (Wobeser 2002; Illinois Department of Natural Resources 2010). Some states have less intensive management programs, although effort is difficult to quantify. CWD continues to spread and prevalence in some managed areas is increasing (Wisconsin Department of Natural Resources 2010). Because environmental characteristics influence disease dynamics, they could also influence the effectiveness of management strategies (Cullingham *et al.* 2008). More information on the effect of landscape on prion disease epidemiology and cervid ecology is required to evaluate disease control strategies and optimize their effectiveness.

Molecular genetic techniques provide a tool for examining deer movement and CWD and host mediated prion spread. The distribution of genes across the landscape can be used to measure gene flow, an indicator of deer movement (Bohonak 1999; Berry *et al.* 2004). By understanding how cervids move across the landscape, we can better predict CWD spread via deer dispersal. We predict that if disease occurrence is found in areas that show high gene flow, then deer movement could be enhancing the spread of CWD. On the other hand, elevated CWD prevalence in areas with reduced gene flow, could indicate that philopatric behaviors increase the risk of CWD exposure, thereby facilitating disease spread (Hess 1996).

Several statistical approaches for quantifying gene flow are available, and they vary widely in function and complexity. Basic fixation indices like F_{ST} can be used to examine genetic differentiation (Wright 1943), while more complex Bayesian algorithms like assignment tests can quantify population structure and genetic admixture (Pritchard *et al.* 2000). In this study we used a variety of statistical methods to analyze microsatellite genotypes of white-tailed populations in southern Wisconsin and Illinois. CWD was detected in the sampled population in 2002, first in south eastern Wisconsin and then along the Wisconsin-Illinois border (Illinois Department of Natural Resources 2010; Wisconsin Department of Natural Resources 2010). Currently, it is not clear if these outbreaks are independent events, but spatial heterogeneity of CWD prevalence in these areas (Osnas *et al.* 2009) suggests spread may be affected by habitat or deer movement. Hence, our goal was to examine deer movement and population structure in the context of CWD transmission and spread. To accomplish this we addressed several objectives:

1. Quantify population level movements, admixture and gender-biased dispersal patterns using F_{ST} and contingency tests.
2. Examine movements of individuals using assignment tests and spatial autocorrelation analyses.
3. Quantify dispersal of individuals using parentage assignment.
4. Compare genetic characteristics such as allelic diversity, heterozygosity and fixation indices between CWD infected and uninfected individuals to determine if CWD is associated with movement of white-tailed deer.

These objectives are addressed using a variety of analytical methods to examine population and individual level data. This approach allows us to gain a comprehensive view of genetic patterns and movement of white-tailed deer which will add to our knowledge of CWD dynamics.

Materials and Methods

Deer Sampling

We utilized tissue samples collected through CWD surveillance and population control programs in Illinois and southern Wisconsin. The majority of samples were collected from areas at increased risk for disease based on proximity to prior CWD cases. Additional samples were from areas of special management interest. Harvest date, gender, age and spatial locations were collected for all samples.

North-central Illinois sampling area (NIL). Between January 2003 and March 2009, Illinois Department of Natural Resources (IDNR) harvested ~5,000 free-ranging deer in the CWD-infected region of northern Illinois (Fig. 6.1). Of the samples collected, 921 from Winnebago, Boone, DeKalb, Ogle, and McHenry counties were used for genetic analysis. All samples collected in 2003, 2004 and 2006 were selected for genetic analysis, in addition to any samples that tested positive for CWD. Using samples from the early years should enable us to test if our predictions of disease spread indeed match the current distribution of CWD in NIL. For deer sampled in NIL, spatial locations were recorded to the nearest township/range/section (TRS; 2.6 km^2).

North-western Illinois sampling area (GTA). Between January and February 2008, 219 free-ranging deer were harvested in population control programs from Galena Territory Association in JoDaviess County (Fig. 6.1). These samples provided an opportunity to analyze uninfected populations to the west of the CWD outbreak. For deer sampled in GTA, spatial locations were recorded to the nearest 0.1 km.

North-eastern Illinois sampling area (DuP). Between December 2007 and January 2008, 50 free-ranging deer were harvested by United States Department of Agriculture Wildlife Services

personnel through deer management programs initiated by the DuPage County Forest Preserve (Fig. 6.1). Samples collected in DuP allowed us to analyze uninfected populations to the east of the CWD outbreak. For deer sampled in DuP, spatial locations were recorded to the nearest township/range/section (TRS).

East-central Illinois sampling area (RAP). During fall hunting seasons between 2005 and 2007, 319 free-ranging deer were harvested at University of Illinois Robert Allerton Park (RAP) through their Deer Management and Research Program (Fig. 6.1). Samples collected in RAP allowed us to describe genetic structure in at a larger spatial scale. To date, CWD has not been detected in RAP. For deer sampled in RAP, spatial locations were recorded to the nearest km.

Wisconsin sampling Area (WI). Between October 2003 and March 2006, Wisconsin Department of Natural Resources (WDNR) collected samples from 71,611 free-ranging deer harvested by hunters in the CWD management zone in southern Wisconsin. Of the samples collected, 479 from Grant, Lafayette, Green, Rock, Walworth, Racine, and Kenosha counties were used for genetic analysis (Fig. 6.1). Deer in WI were selected from harvests conducted in 2002 and 2006 in an attempt to match the age distribution of deer sampled in NIL. We selected sampling areas in WI located at varying geographic distances from sampling areas in Illinois so that the effects of geographic distance on genetic structure could be examined. For deer sampled in WI, spatial locations were recorded to the nearest township/range/section (TRS).

Laboratory Procedures

Muscle samples were stored in 100% ethanol and genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison WI). Individuals were genotyped using 10 microsatellite DNA primers previously described in Kelly *et al.* (2010). Forward primers were labeled with fluorescent dyes (NED, HEX, FAM) with fragments separated on an ABI 3730XL

capillary sequencer (Applied Biosystems, Foster City, CA) and visualized with GeneMapper (v. 4.0: Applied Biosystems, Foster City, CA). MicroChecker (v.2.2.3; Van Oosterhout *et al.* 2004) was used to evaluate genotyping errors using expected allele frequencies derived under Hardy-Weinberg Equilibrium (HWE).

Descriptive Statistics

We used Arlequin (ver 3.0; Excoffier *et al.* 2005) to calculate observed (H_o) and expected (H_e) heterozygosities for each locus and average expected heterozygosity for each sampling area. We used GenePop (ver. 4.0; Rousset 2008) to calculate the number of alleles per sampling area (allelic diversity), the number of rare alleles (alleles exclusive to one sampling area) and deviations from HWE. Hardy-Weinburg Equilibrium was examined by locus, by sampling area and overall with significance determined using 10,000 dememorization steps, and 5000 iterations in each of 100 batches.

Population-Level Movements

Since the majority of deer were sampled in NIL and WI, these areas were subdivided into 28 study sites based on geographic proximity of the samples. To the best of our ability, we tried to allocate approximately equal numbers of individuals into each study site (~30 deer). For clarity we will subsequently refer to GTA, RAP, and DuP as study sites as they will be analyzed with the 28 study sites from WI and NIL (31 study sites total). We calculated a matrix of pairwise F_{ST} values for all 31 study sites in Arlequin (ver 3.0). To determine if gene flow was restricted by geographic distance we tested for isolation by distance (IBD). To test for IBD, we correlated the matrix of pairwise F_{ST} values with a matrix of pairwise geographic distances (km) using Mantel tests in GenAlEx 6.2 (Rousset 2008). One-thousand random permutations of the observed data

were used to examine correlations for significance. When performing tests for IBD, we analyzed both genders combined, then each separately.

To define genetic population boundaries, our study sites were coalesced into populations based on homogeneity of allele frequencies determined by contingency tests in TFGPA (v.1.3; Miller 1997). A significant contingency test ($P<0.05$) indicates that allele frequencies between study sites are genetically heterogeneous, while a non-significant test indicates that allele frequencies between study sites are not different, and study sites are genetically homogeneous. To approximate probabilities associated with observed allele frequencies for each locus, pairwise χ^2 tests were calculated from contingency tables of allele frequencies for all pairwise combinations of study sites. Significance was evaluated using Markov Chain Monte Carlo (MCMC). For each single-locus test, 5000 permutations were completed for each of 20 batches with 1000 dememorization steps. *P*-values from single-locus tests were pooled using Fisher's Combined Probability Test and multi-locus *P*-values (<0.05) were evaluated for departures from homogeneity in allele frequencies between populations. To prevent multi-locus tests from being dominated by a single marker, single-locus *P*-values were minimized at $P = 0.0001$ (Waples and Gaggiotti 2006).

Study sites not significantly different from one another in the contingency tests were then coalesced as samples representing the same genetic population. Populations consisted of either one site for which all other multi-locus tests were significant, or instead as multiple study sites linked through a series of non-significant tests (Waples and Gaggiotti 2006).

To determine if female or male dispersal contributed to homogenization of allele frequencies among our 31 study sites, we tested for sex-biased dispersal in the populations defined through contingency tests using FSTAT (v.2.9.3.2; Goudet 2001). Gender specific

dispersal was tested by determining significance of F_{ST} for males and females across study sites. We calculated male and female F_{ST} values separately and evaluated significance using 1000 random data permutations. Two separate analyses were performed: one with genetically distinct populations (study sites for which all multi-locus tests were significant; 7/31 sites) and a second on the study sites which were linked into a single population through a series of non-significant tests (24/31 sites).

Individual Movements

Bayesian Assignment of Individuals

We inferred population structure using a Bayesian method to assign individuals to genetically similar clusters. Bayesian clustering was implemented in STRUCTURE (v. 2.3.1; Pritchard *et al.* 2000) which estimates the natural logarithm of the probability [LnP(D)] that individual genotypes belong to a given cluster (*k*=number of clusters to which individuals are assigned during analysis). Assignment of individuals eliminates the need for *a priori* population definitions. Twenty replicates were run for *k* values 1 through 7 and the simulation yielding the smallest Bayesian deviance was selected as the optimal model for the number of clusters (Pritchard *et al.* 2000; Faubet *et al.* 2007).

The Bayesian method implemented in STRUCTURE can incorporate background information about the population to better examine admixture. An admixture model (initial α =1.0, max α = 10.0, SD of α = 0.05) was computed with TRS as the location for each deer and correlated allele frequencies specified to account for shared ancestry (Pritchard *et al.* 2000; Falush *et al.* 2003). To facilitate interpretation of results, study sites were specified as the population identifiers for STRUCTURE, so their respective F_{ST} values could be compared to simulated admixture proportions. Initially, several pilot simulations were run to determine burn-

in length and consistency of parameter estimates across replicates. Male and female clusters were simulated together and separately using a burn in of 100,000 MCMC steps followed by 100,000 replicates to estimate posterior probabilities of all parameters. Individuals were assigned to the inferred cluster containing the highest percentage of membership (q).

Spatial Autocorrelation

Global spatial autocorrelation explores spatial structuring across the entire sampled region by examining correlations between geographic and genetic distance matrices at varying spatial scales (Sokal and Oden 1978). Populations will exhibit positive spatial autocorrelation that declines with increasing distance if relatives are spatially adjacent (Smouse and Peakall 1999). Tests for global spatial autocorrelation were performed with individuals from NIL and DuP using the single population procedure in GenAlEx (ver. 6.2; Peakall and Smouse 2006). Deer from GTA and RAP were omitted because of inadequate sampling at intermediate distances in these locations. Individuals were analyzed separately by gender and age class (fawn, yearling or adult; Table 6.2) so that sex-biased dispersal and juvenile movements could be examined. For all individuals, pairwise geographic distances were calculated from the x/ y-coordinates of the TRS centroid from which they were sampled. Pairwise geographic and squared genetic distance matrices were used to calculate *r*, the autocorrelation coefficient for all individuals in each gender-age class at distances ranging from 0.5-100 km. A one-tailed distribution was derived from 999 random permutations and bootstrapped to determine significance and 95% confidence limits. Distance was divided into classes for analysis in order to examine genetic structure at increasing spatial scales. Classes were 0-0.5 km (within a TRS), 0-2 km (between adjacent TRS), 0-3 km (separated by 1 TRS), 0-6 km, 0-12 km, 0-24 km (~ 1 dispersal event; Nixon *et al.* 2007), 0-48 km, 0-100 km (long-distance dispersal).

To further examine spatial autocorrelation of individuals, two dimensional local spatial autocorrelation analyses were conducted in GenAlEx (ver. 6.2) Tests for local autocorrelation can detect patterns at a finer resolution than global tests, because autocorrelation coefficients (*r*) are calculated for each individual and its nearest neighbors, rather than for groups within a given distance class (Sokal *et al.* 1998). With local tests, varying numbers of nearest neighbors can explored to determine how genetic patterns change as more individuals are added to the group. In addition to the gender-age classes examined in global analyses, adult females and fawns of both sexes were grouped and analyzed, and adult males and female yearling were grouped and analyzed. This was done based on the results from the global analyses indicating strong autocorrelation in females and fawns, and a general lack of autocorrelation in adult males and female yearlings. Five, 10, 15, 20 and 25 nearest neighbors were examined for all groups analyzed using 999 conditional permutations. We focused on results from five, 15, and 25 nearest neighbors because we were interested in the consistency of autocorrelation patterns for variable group sizes. Also, examining 25 nearest neighbors facilitates the identification of locations supporting large family groups.

To determine statistical significance of the correlation, the observed *r* was compared to a distribution of *r* values generated through conditional permutation. Conditional permutation fixes the spatial coordinates for the pivotal observation and shuffles coordinates for all other individual (Peakall and Smouse 2006). To account for multiple samples in the same TRS, *r* values were ranked based on conditional *P*-value and the strength of the correlation (the strongest, most significant *r* value received a rank of 1). Rank values were then averaged for each TRS (Sokal *et al.* 1998), and the top 10% of TRS were mapped to visualize spatial patterns.

Parentage Assignment

Tests for parentage assignment were performed to quantify dispersal of individuals from their mothers. Mother-offspring pairs were identified using Cervus 3.0 (Kalinowski *et al.* 2007). Cervus employs a multi-step process to first simulate the distribution of log-likelihood values for mother-offspring pairs using allele frequency data from sampled populations, and second determine statistical confidence of parentage for sampled individuals. To ensure conservative parentage estimates, an error rate of 0.001 was used (observed error rate= 0.005, data not shown) in simulations of 100,000 offspring. All females were used as candidate mothers while all individuals were considered potential offspring with subsequent removal of duplicate parentage assignments for a single pair of individuals. We validated the ability of our markers to resolve parentage by including 100 confirmed mother-offspring pairs (mothers with fetuses *in utero*). Based on simulations of the observed data and validation with mother-offspring pairs assignments at 95% confidence were evaluated.

To measure dispersal, spatial distances between mother-offspring pairs were calculated with the geographic distance calculation in GenAlEx (ver. 6.2). While individuals from all sampling areas were included in the parentage analysis, mother-offspring pairs identified within RAP were not used to calculate dispersal distances because most RAP individuals were harvested within <2 km. Individuals from NIL, DuP, and GTA that paired with a RAP individual were, however, included in the analysis.

Genetic Evaluation of CWD Infected Deer

To determine if CWD infection influenced deer movement behavior, we used FSTAT (v.2.9.3.2) to compare genetic characteristics of CWD positive (*CWD+*) versus CWD negative (*CWD-*) deer in NIL and WI. For this analysis individuals from RAP, GTA, and DuP were omitted because

CWD has not been detected in these areas. For deer in WI and NIL, we first calculated average values of allelic richness (using rarefaction index to account for differences in sample size), H_o, H_e, F_{IS}, F_{ST} and relatedness for *CWD+* and *CWD-* deer. Next, these values were evaluated for significant differences using a two-tailed test [$OSx = \sum_{i=1}^{(\#ofgroups-1)} \sum_{j=i+1}^{(\#ofgroups)} (x_i - x_j)^2$; where x=genetic test statistic; *i*=group 1(*CWD-*); *j*= group 2 (*CWD+*)] based on a distribution of estimates generated from 10,000 random permutations (Goudet 2001). Since randomization in FSTAT is performed at the group level, we first tested for significant differences using two groups (*CWD+* and *CWD-*) and then randomly partitioned *CWD+* and *CWD-* deer into 30 groups (15 for *CWD+* and 15 for *CWD-*). We then retested for significant differences in the aforementioned statistics between *CWD+* and *CWD-* groups.

Results

Descriptive Measures

Multi-locus genotypes were obtained for ~2,000 deer across 5 sampling areas in the northern half of Illinois and southern Wisconsin. None of the 10 microsatellites violated HWE assumptions, suggesting that null alleles and large-allele drop-out did not affect genotype scoring. However, one marker in NIL (RT23) showed significant heterozygote deficiency, but F_{IS} was low (F_{IS} = 0.018) suggesting subpopulation structure rather than inbreeding. Global tests showed no evidence of heterozygote excess in any of the sampling areas. Number of alleles per locus (average = 11) ranged from 2 (OarFcb304) to 19 (RT23), with 9 rare alleles observed across 4 loci in NIL, RAP, and WI. Sample sizes, observed heterozygosity, and number of alleles, both common and rare to one sampling area are shown for NIL, GTA, DuP, RAP and WI in Table 6.1.

Population-Level Movements

IBD was not detected when individuals from all 31 study sites tested (Mantel r =, *P*=), nor when male and female deer were analyzed separately, indicating that gene flow was not influenced by distance or that historical anthropogenic influences have shaped genetic structure. Contingency tests revealed vast differences in genetic heterogeneity for study sites within the five study areas (Fig. 6.2). Sites with a greater number of significant tests were more genetically distinct from other sites. The number of significantly different tests per study site ranged from 0 to 16. Seven (of 31; 22.6%) study sites [31 (RAP), 13 (GTA), 14, 15, 18, 19 and 24] were significantly distinct populations ($P<0.05$). The remaining study sites in DuP, NIL and WI were linked through a series of non-significant tests, thus indicating a single genetic population resulting from extensive gene flow as indicated by non-significant contingency tests (Fig. 6.2).

Figure 6.2 also shows the number of non-significant contingency tests per study site (represented by the height of grey bars) in relation to the number of positive individuals (represented by the height of red bars) detected at those sites between 2002 and 2009. A few of the sites with a relatively greater number of CWD cases (sites 19, 22, 23) were genetically heterogeneous often having fewer than two non-significant tests. In contrast, many sites with a few cases of CWD (sites 20, 25, 26, 28) were much more genetically admixed as they tended to have several non-significant contingency tests.

Gender-biased dispersal was not detected when all 31 study sites were analyzed together (female F_{ST}=0.004; male F_{ST}= 0.003; *P*=0.4). However, when the seven genetically distinct populations [31 (RAP), 13 (GTA), 14, 15, 18, 19 and 24] identified by contingency tests were subsampled, significant male-biased dispersal was detected. The average F_{ST} value for females in these seven populations was higher than the value for males (female F_{ST}=0.020; male F_{ST}=

0.013; *P*=0.02), indicating females are more genetically structured than males. When the 24 genetically homogeneous study sites were analyzed, gender-biased dispersal was not detected (female F_{ST}=0.004; male F_{ST}= 0.003; *P*=0.4).

Individual Movements

Bayesian Assignment of Individuals

When all deer were analyzed, five clusters produced the lowest Bayesian deviance value (Fig. 6.3a). All five were in HWE (Bonferroni corrected P<0.001), although membership for some study sites was proportioned across multiple clusters. A simulation with four clusters produced the second lowest Bayesian Deviance value. The five cluster model placed GTA and RAP into separate clusters and divided NIL and WI into three clusters; one spanning the southern border of Wisconsin, one to the northeast of Rockford, and one to the southwest of Rockford. The RAP study site was more genetically distinct (at 90% membership). Deer from GTA were less distinct than RAP, but more distinct than the other clusters with 66% of membership assigned to their respective cluster.

For study sites in the WI, NILNE and NILSW clusters, admixture was high along contact zones, and for a few study sites, membership was more or less evenly divided between multiple clusters (eg. 29, 30), indicating extensive gene flow along the exterior of the inferred populations. This is best illustrated in Fig. 6.3c, which shows a gradual decrease in individual q values (proportion of individual membership into clusters) for inferred clusters after they were sorted largest to smallest. While some deer in WI, NILNE, and NILSW had high cluster membership, most had mixed membership with other clusters indicating migrant ancestry. For these clusters, boundaries are more representative of contact zones between different gene pools than population delineations. This is in contrast to RAP and GTA where most deer had high

cluster membership, with a small proportion of deer demonstrating mixed ancestry with the other clusters.

The observed variation in admixture can be seen on a finer spatial scale in Fig. 6.4 which shows individual q-values (each individual's proportion of membership in their assigned cluster) averaged for each TRS. At this spatial scale it appears that most TRS had low average q values, indicating recent migrant ancestry and high admixture. However, there were a few areas (RAP, GTA, near study sites 19, 23, 14 and 24) where individual membership was high, indicating that individual genotypes were consistent with the resident gene pool of the inferred cluster. Those TRS with high membership coincided with study sites that were genetically distinct according to contingency tests indicated in Fig. 6.2.

When females were analyzed separately, five clusters produced the lowest Bayesian deviance value (Fig. 6.3d). This allocation for female deer was consistent with the model selected when all deer were analyzed together with a couple of exceptions. GTA was assigned to a separate cluster, as observed with the model for all deer. However, in contrast to the model with all deer, RAP and DuP were assigned to the same cluster. In females, the same study sites were assigned to NILSW as in the overall model, with the omission of DuP. The third cluster was consistent with the NILNE cluster detected in the overall model, but study sites 5, 6 and 20 were omitted from this cluster for females. For female deer study sites 5, 6 and 20 were combined with sites to the north, rather than to the south as for all deer, forming a cluster that spanned the southern border of WI (Labeled WI in Fig. 6.3d; compare to location of these sites in NILNE for all deer in Fig. 6.3b).

For females, the RAP study site was the most genetically distinct (at 90% membership), whereas deer from GTA were less distinct with 51% of membership assigned to their respective

cluster. Females in the WI cluster averaged 39% membership and females in the NILNE and NILSW clusters averaged 41% and 47% membership respectively. All inferred female clusters were in HWE (Bonferroni corrected $P<0.001$).

When males were analyzed separately, four clusters produced the lowest Bayesian deviance value (Fig. 6.3e). The model for males placed GTA and RAP into separate clusters and divided NIL and WI into two clusters; one including all WI study sties and all study sites to the north, east and west of Rockford, and another cluster including all study sites to the south of Rockford and DuP (Fig. 6.3). The RAP study site was the most genetically distinct (at 81% membership), whereas deer from GTA were less distinct with 44% of membership assigned to their respective cluster. The remaining study sites averaged 46% membership and tended to be admixed with adjacent clusters. All inferred male clusters were in HWE (Bonferroni corrected $P<0.001$).

Spatial Autocorrelation

In global autocorrelation analyses, adult males were not spatially structured at distances up to 100 km, as 95% bootstrap confidence limits overlapped 0. However, male yearlings were structured at 0-3 km, 0-6 km, and 0-12 km (Fig. 6.5) as indicated by *r* values greater than 0. At ≤0.5 km, male fawns demonstrated the strongest positive spatial autocorrelation of any group examined, averaging $r=0.035$ for this distance class. For male fawns, significant autocorrelation was maintained at distances ≤3 km, then declined sharply. Adult females also showed positive spatial autocorrelation that was maintained at distances ≤48 km, with gradual decreases in *r* observed with increasing distance between 0 and 48 km (Fig. 6.5). Female yearlings were not spatially autocorrelated at any distance, and average *r* values were similar to those observed in

adult males. Like male fawns, female fawns were also positively autocorrelated, though female fawns showed significant structure at larger distances (≤6 km) than males.

With the exception of adult males and male fawns, local autocorrelation analyses using different number of nearest neighbors showed that the percentage of significant local *r* values increased as additional neighbors were included. However, average *r* values decreased with additional nearest neighbors (Table 6.3). For the same number of nearest neighbors, average local *r* values were similar across groups, although maximum *r* values tended to be higher for females, especially adults.

When local *r* values were ranked and ranks were averaged, adult females and fawns showed several TRS with significant autocorrelation for 25 nearest neighbors. The lowest ranked (top 10% of rank scores) TRS for adult female and fawn clusters are shown in Fig. 6.6, along with the two locations yielding the highest (top 1%) individual *r* values observed for this group. Adult males and female yearlings had far fewer significant *r* values with 25 nearest neighbors, and correlations were substantially weaker than those for adult females and fawns. In fact, none of the correlations for this group were >0.10. In Fig. 6.7 (top panel), we have shown TRS where males and female yearlings exhibited low rank scores, usually attributable to a single, weak but significant *r* (Fig. 6.7, bottom panel). Interestingly, in adult females and fawns, and adult males and female yearlings, TRS along the Winnebago-Boone County border demonstrated low rank (and high individual correlations scores for adult females and fawns). This area coincides with a CWD hot spot in Illinois.

Uneven sampling can influence patterns of local autocorrelation (Double *et al.* 2005), as correlations can be more readily detected with increased sampling intensity. In this study, our sample sizes for each group at each of the nine distance classes, shown in Table 6.2, were not

equal which may have influenced autocorrelations. However, although some of the locations that were autocorrelated were in areas that were sampled heavily, the majority were not. Further, rank scores were not correlated with the number of deer sampled in each TRS for adult males and female yearlings (r=0.04, P=0.59), and rank was only weakly correlated with sample number in adult females and fawns (r=-0.16, P=0.03).

Parentage Assignment

Simulations of parentage using allele frequencies of the sampled population showed that only 1% of non-mothers were assigned maternity at 95% confidence when the true mother was not sampled, and 4% when the true mother was sampled. All mother-fetus pairs used for validating parentage were correctly assigned at 95% confidence levels. For individuals with unknown parentage, Cervus assigned 49 mother-offspring pairs at 95% with no mismatches across all multilocus genotypes. Of the 49 pairs assigned, 34 were mother-daughter pairs and 15 were mother-son pairs. All assigned male offspring were ≤ 3 years old, with 67% of mother-son pairs being fawns. Twelve mother-daughter pairs included fawns (35%), four included yearlings (12%), and the remaining 18 assignments were between adult females (53%). Over two-thirds (73%) of the mother-offspring pairs detected were within the same home range (as estimated by Nixon *et al.* 1991 for deer in RAP).

Of the 49 parentage assignments made, four involved individuals infected with CWD. For one of these pairs, the adult mother and her female fawn were both infected with CWD. The second and third pair included adult females, one pair with an infected mother, and the other pair with an infected daughter. The last pair included an infected male fawn and his uninfected adult mother.

Dispersal distances for assigned pairs ranged from 0 to 194 km with an average distance of 27 km (Fig.8). In total, there were 17 pairs that were separated by <1 km, and of these, 14 were mother-fawn pairs. The assigned pairs that involved CWD infected deer tended to be at the lower end of the dispersal distance distribution. Three of these pairs had dispersal distances <5 km, though one mother-daughter pair assigned at 95% confidence had a dispersal distance of 135 km. The shape of the distribution of dispersal distances is similar to the distribution of distances between CWD infected TRS, as both are highly skewed towards low values (Fig. 6.8).

Genetic Evaluation of CWD Infected Deer

Overall, estimates of allelic richness, H_o, H_e, F_{IS}, F_{ST} and relatedness were similar between *CWD+* and *CWD-* deer in all of the groups analyzed. There were no significant differences detected in any of these estimates when two groups (1 *CWD+* and 1 *CWD-*) or 30 groups were analyzed (15 *CWD+* and 15 *CWD-*).

Discussion

According to our estimates of genetic admixture and gene flow, white-tailed deer move extensively in the heterogeneous habitats of southern Wisconsin and northern Illinois. Contingency tests indicated that gene flow had homogenized allele frequencies for deer across >240 km along the Wisconsin-Illinois border (Fig 2). Further, admixture along genetically defined clusters suggested substantial gene flow between study areas. Consistent with this implied gene flow, Bayesian assignment tests revealed that population structure was generally weak across the study area, especially in southern Wisconsin and near Rockford, Illinois (Fig. 6.3). This level of admixture across this large of an area seems unusual. Deer in Kentucky (Doerner *et al.* 2005), Michigan (Blanchong 2003), South Carolina and Georgia (Purdue *et al.*

2000) exhibited higher genetic differentiation according to F_{ST} than the deer we studied in Illinois and southern Wisconsin.

Our genetic data supports the hypothesis that deer movement in our study area can facilitate CWD spread (Hess 1996). Of additional concern, we observed evidence of long distance dispersals involving CWD infected deer. With parentage analysis, we detected a CWD infected female sampled 135 km away from her mother. If deer disperse after they become infected, they can directly transmit CWD when they come in contact with other deer. Usually a dispersing female would not be accepted into an unrelated female matriarchal group, and so direct contact with other unrelated females would be unlikely (Oyer *et al.* 2007). However, it is possible that a dispersing male would be accepted into a bachelor group. Males in bachelor groups participate in grooming behaviors (Marchinton and Hirth 1984) which could transmit infected prions through saliva (Mathiason *et al.* 2006).

Alternatively, sick animals, especially those that are clinically ill, can shed infectious prions into the environment and contaminate the areas they traverse (Williams and Miller 2002). During the rut, male deer often scrape the ground and then urinate, or rub branches with their antlers to mark territories (Gutschow 2005). Urine (Gonzalez-Romero *et al.* 2008) and antler velvet (Angers *et al.* 2009) can contain infectious prions, and so these behaviors could be facilitating environmental transmission of CWD, especially since multiple deer often visit rubs and scrapes within a season (Marchinton and Hirth 1984; Gutschow 2005).

Although IBD, contingency tests, and assignment tests revealed that gene flow and admixture was generally high for deer in our study area, there appears to be a few sites in northern Illinois and southern Wisconsin where gene flow is drastically reduced. Sites in JoDaviess County (GTA), southern Ogle County (site 14), northwestern DeKalb County (site

24), and along the Winnebago-Boone County border (study sites 19, 23) showed reduced admixture with contingency tests and assignment tests. In these same areas, female philopatry was apparent according to tests for sex-biased dispersal and local spatial autocorrelation analyses (Fig. 6.2, Fig. 6.3, Fig. 6.4 and Fig. 6.7). These findings are important because sites 19, 23, and 24 are within or adjacent to CWD hotspots in Illinois that have maintained the highest disease prevalence (Illinois Department of Natural Resources 2010).

Deer behavior and movement are known to be influenced by habitat fragmentation and urbanization and sites 19 and 23 are located to the northeast of Rockford, in fragmented habitats that contain patches of forest and a nature preserved intermixed with residential development. Deer density in this area is slightly higher than the average deer density in northern Illinois (sites 19, 23=6.2 deer/mi^2, average for areas surveyed in 2009 in northern Illinois=5.7 deer/mi^2; Illinois Department of Natural Resources 2010). Farnsworth *et al.* (2005) found that CWD prevalence in mule deer was higher in developed habitats than in undeveloped habitats. The authors attributed this difference to local factors such as replenishable food sources, and smaller habitat fragments in developed areas that concentrate deer in one location (Farnsworth *et al.* 2005; Wolfe *et al.* 2002). The habitat (urban, fragmented) and philopatric behaviors of female deer in sites 19 and 23 could be increasing deer density and CWD transmission, thereby creating a hotspot for disease.

Site 14 in Ogle County, like sites 19 and 23, showed strong evidence for reduced gene flow according to contingency tests, Bayesian assignment tests, and local spatial autocorrelation analyses. In fact, this site in Ogle County had the lowest rank and highest *r* values for females and the lowest rank for males, suggesting that deer in this location tend to be philopatric. In contrast to the habitat near Rockford, the undeveloped habitat in this area of Ogle County

consists of a state park and nature preserve with large patches of forest along a riparian corridor. Deer density in this area is much higher than the average deer density in northern Illinois (site 14=15.2 deer/mi^2, average for areas surveyed in 2008 in northern Illinois=5.7 deer/mi^2; Illinois Department of Natural Resources 2010). Only a single case of CWD has been detected here despite several years of continued surveillance (Illinois Department of Natural Resources 2010), perhaps because the habitat patch is larger and more continuous which could deter deer movement to other locations and increase local density (Long *et al.* 2005). Alternatively, increased hunting pressure in Ogle County (compared to other counties in IL, data not shown) and proactive disease surveillance at site 14 could have prevented CWD from becoming established by removing CWD infected individuals before they began shedding infectious prions (Williams and Miller 2002). Nevertheless, the philopatric behavior of deer in sites like Ogle County combined with high deer density would pose a risk for accelerated transmission of CWD via direct contact between female relatives (Farnsworth *et al.* 2005). It is our recommendation that disease surveillance in sites demonstrating reduced genetic admixture and increased density (eg. GTA, Ogle County) continues, to prevent the establishment of additional disease foci.

Areas of high admixture represent areas with greater potential exposure to CWD. There was spatial variability in genetic admixture which appears to be mostly attributable to differences in female philopatry in some areas. This variability is the result of low movement by females (high philopatry) relative to other areas. The finding that males and females disperse across the majority of the study area has implications for CWD. In the CWD outbreak in south central, Wisconsin, CWD prevalence is higher in males than females, suggesting greater potential transmission resulting from male behaviors (Grear *et al.* 2006). This difference has not been observed in Illinois deer (Illinois Department of Natural Resources 2010). However, F_{ST} values

for females in our study areas, which included the southern regions of Wisconsin as well as northern Illinois, tended to be lower than for deer located in the northern portion of the CWD outbreak area (south central Wisconsin, near Mt. Horeb; Blanchong *et al.* 2008; supplementary data, Table 6.1). Also, female philopatry has been reported in the south central Wisconsin CWD outbreak (Skuldt *et al.* 2008), so females near the CWD foci in Mt. Horeb, Wisconsin appear to have reduced movement or dispersal compared to females in our study area. Dispersal could become a risk factor for CWD in several different ways: Females or males dispersing from non-infected locations into infected locations would experience increased exposure to disease compared to non-dispersing deer. Conversely, increased deer dispersal from infected areas could potentially spread disease to a larger area. Hence, dispersal could have attributed to similar CWD exposure and disease prevalence in female and male deer in our study area.

We did not detect differences in genetic characteristics (relatedness, H_o, H_e allelic richness or fixation indices) between *CWD+* and *CWD-* deer suggesting that CWD infection is not associated with alteration in dispersal, movement or related behaviors. However, given the fact that behavioral changes are associated with late-stage clinical illness (Williams and Miller 2002), this result is not unexpected since most of the positive deer in our study did not exhibit any overt clinical signs of disease when harvested. As infectious prions accumulate in the nervous tissue of CWD infected deer, clinical signs including reduced alertness, isolation (Williams and Young 1980) and a loss of fear (Spraker *et al.* 1997) result, and some studies have reported behavioral differences in CWD infected deer. Krumm *et al.* (2005) reported that CWD infected deer were more susceptible to death by vehicle collision than uninfected deer. Further, CWD infected deer in Colorado were more prone to predation by mountain lions than uninfected deer (Miller *et al.* 2008). It is possible that in both of these cases, behavioral changes occurred at

later stages of disease progression, while in the current study, deer were sampled across the population so infected animals would be expected to represent a range of clinical progression. Reduced migration and dispersal has also been reported in *CWD+* deer compared to *CWD-* deer (Edmunds 2008). In our study we detected four mother offspring pairs in which one individual was *CWD+* and the infected individuals were separated by <5 km. This was much smaller than the average distance of 27 km between all pairs. In fact, in one of these pairs, both mother and daughter were *CWD+* and they were harvested in the same TRS in sequential years. Though the number of our observations is very low to draw firm conclusions, these results clearly support the findings of Edmunds (2008) that CWD causes reduced movements. Since it is likely that deer in our study represent individuals with a range of progression toward clinical disease, this suggests the behavioral changes related to movement occur relatively early compared to other clinical signs. Alternatively, genetic polymorphisms in the prion protein which are related to CWD susceptibility (Kelly *et al.* 2008) and expressed in the brain, may be related to altered movement behavior regardless of disease status.

Surprisingly, we detected significant positive autocorrelation in male yearlings, while female yearlings were not autocorrelated. These findings are unexpected because most studies report male yearling dispersal (Nelson and Mech 1984; Nixon 1991) and female yearling philopatry (Hawkins and Klimstra 1970; Mathews and Porter 1993). It has also been reported that females tend to share home ranges with their mothers (Hawkins and Klimstra 1970) for up to two years before they disperse (Ozoga and Verme 1984; Ozoga *et al.* 1982), and they usually establish their own home ranges nearby (Mathews and Porter 1993). Our findings suggested that in the region studied, female yearlings had dispersed at the time of sampling (January-March in Illinois, November-December in Wisconsin). Further, global trends for yearling males indicated

autocorrelation up to 12 km, though local analyses revealed that these patterns were influenced by a few strongly positively autocorrelated sites. Differences between direct measures of yearling movement in other studies and our genetic measures could be related to the larger sample size in our study (female yearling N =124, male yearling N=198) compared to other studies (Hawkins and Klimstra 1970: female yearling N=79, male yearling N=179; Mathews and Porter 1993: female N=69, male N=26). Alternatively, the difference might be the ability of genetic analysis to detect rare or cryptic patterns of movement that are difficult to detect in observational studies of practical duration (Allendorf *et al.* 2008).

In this study most deer were sampled from January to March, when they are most likely to be concentrated in winter ranges. Deer tend to reduce their home ranges in winter when resource availability becomes limited (Tufto *et al.* 1996), oftentimes congregating in winter yards with multiple family groups (Marchinton and Hirth 1984). Therefore, the timing of our sampling could have influenced our observations of autocorrelation and philopatry. If winter ranges attract subgroups of larger family units (Marchinton and Hirth 1984), then our ability to detect autocorrelation and reductions in gene flow would have been increased because genetic relatives would be concentrated in one location. Alternatively, winter ranges could draw in deer from multiple family groups which may have resulted in observed patterns of genetic admixture. In either case, winter ranges could alter movement patterns of deer or the distributions of populations, and contribute to CWD spread and transmission.

Unlike experimental studies that are designed to test specific hypotheses, we conducted a genetic survey utilizing samples collected for disease surveillance in Illinois and southern Wisconsin. We realize that this sampling strategy could have introduced bias in our observations. Because samples were collected to detect disease, and not to sample continuously across the

landscape, the likelihood of detecting mother-offspring pairs and spatial autocorrelation was not equally probable in all directions. Hence, while parentage analysis allowed us to detect many mother-offspring pairs separated by short distances, our sampling distribution most likely prevented detection of mother-offspring pairs at larger distances. Further, using this method to detect dispersal assumes that the mother remains philopatric which could be unrealistic for certain areas within northern Illinois and southern Wisconsin. To account for these potential biases, we validated the ability of our markers to resolve parentage by including 100 confirmed mother-offspring pairs (mothers with fetuses *in utero*). We also used 100,000 offspring simulations to ensure that estimated likelihood distributions were precise as recommended by Pritchard *et al.* (2000). Additionally, we used a conservative error rate and only reported parentage pairs assigned at ≥95% confidence. Still, we recommend interpreting the distribution of dispersal distances obtained from parentage assignment as an approximation of population level movements.

Uneven sampling across the study area could have contributed to the observed patterns of genetic structure, especially those inferred from contingency tests of allele frequencies. However, our multifaceted approach utilizing both population and individual based methods allowed us to prevent bias associated with a single technique. By using averaged ranks of individual autocorrelation coefficients, we were able to reduce the bias associated with sampling heterogeneity. Sampling intensity explained only 2.6% of the variation in TRS rank scores for female deer and less than 1% of the variation in rank scores for males. Further, sampling intensity accounted for less than 11% of the variation in average TRS q values inferred using Bayesian assignment tests. While the effects of sampling heterogeneity cannot be ignored, we do

not believe it has produced bias that prevents inferences about genetic structure of white-tailed deer in the CWD managed region of Illinois and Wisconsin.

In wild deer populations, CWD epidemics are capable of reducing survival and population viability (Miller *et al.* 2008; Edmunds 2008). Since deer are keystone herbivores (Waller and Alverson 1997), the impacts of CWD could presumably alter entire ecosystems, especially without management intervention (Miller *et al.* 2008; Gross and Miller 2001). Our molecular study examining CWD dynamics has shown that host movement could influence the transmission and spread of infectious diseases. We were able to demonstrate that CWD prevalence in Illinois is highest in a location experiencing reduced deer movement and female philopatry. This finding implicates direct contact among deer, especially female relatives as an important transmission mechanism for CWD.

Further, locations experiencing genetic admixture, such as those observed in southern Wisconsin and near Rockford, Illinois, are at risk for disease spread attributable to male and female dispersal. In areas where movement is extensive and philopatry is apparently absent, dispersing females would be more likely to encounter infectious prions in the environment rather than through direct contact with unrelated females (Oyer *et al.* 2007). Dispersing males on the other hand, would be at risk for both direct and environmental transmission given the potential for prion exchange in unrelated bachelor groups (Marchinton and Hirth 1984; Mathiason *et al.* 2006). Collectively, our findings suggest that while movement facilitates disease spread, philopatric behaviors can concentrate CWD in certain habitats.

Although we did not directly quantify the effects of specific habitat features on deer movement and CWD, we were able to demonstrate spatial heterogeneity in deer movement and population structure. This is important because it allowed us to characterize movement patterns

in CWD hotspots and show that small habitat patches in developed areas are associated with reduced movement and increased CWD transmission. Additionally, our findings have shown that locations like site 14 in Ogle County or GTA could be at risk for CWD establishment because they support philopatry and are located in areas adjacent to areas with extensive movement and CWD. Therefore, we recommend that managers continue surveillance in Ogle County and GTA.

Also, areas to the east of the CWD outbreak in Illinois, such as DuP should be monitored. These areas are at risk for prion exposure from dispersing deer, because they exhibit substantial admixture with the infected population. We suggest that additional locations be sampled for genetic analysis, especially those to the south of our study area. Although RAP currently appears to be at limited risk for an influx of dispersers from the infected region in Illinois and southern Wisconsin, our study provided evidence that genetic exchange occasionally occurs. Moreover, the observed tendency for a southernly spread of CWD demonstrates the need to determine the levels and extent of genetic admixture over a larger geographic area.

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Table 6.1. Sample size (*n*), observed heterozygosity, and number of alleles (both common and rare to one sampling area) detected in deer.

Study Site	*n* Females	*n* Males	*n* CWD+	Observed Heterozygosity*	Number of Alleles**	Number of Rare Alleles**
DuP	30	19	0	0.72	87	0
NIL	552	365	103	0.71	119	4
GTA	148	59	0	0.71	99	0
RAP	222	75	0	0.72	113	4
WI	240	238	38	0.71	117	1

*Averaged across 10 loci. Sampling areas did not deviate from HWE, thus expected heterozygosities were nearly identical and not reported. ** Summed across 10 loci.

Table 6.2. Sample sizes (*n*) and number of pairwise comparisons at each distance class for global spatial autocorrelation of deer grouped by gender and age (M=male, F=female) in northern Illinois (NIL), DuPage County (DuP), and Wisconsin (WI).

Age-Gender Class	***n***	**Distance Class**							
		0-0.5	**0-2**	**0-3**	**0-6**	**0-12**	**0-24**	**0-48**	**0-100**
Adult M	229	301	423	559	1090	1950	3818	9797	20508
Yearling M	198	131	301	481	892	1453	2674	7110	14081
Fawn M	194	426	784	1342	2853	4064	8362	14528	18549
Adult F	536	1765	2867	4537	8429	13080	25762	64201	122900
Yearling F	124	40	107	205	428	718	1280	3121	5721
Fawn F	164	352	658	1076	2190	3095	5623	9950	13192

Table 6.3. Percent of significant ($P<0.05$) local r, range of local r, and mean local r for five, 15 and 25 nearest neighbors in groups of white-tailed deer from WI, NIL, and DuP.

Age-Gender Class	Number of Nearest Neighbors								
	5			15			25		
	% $P<0.05$	Range[1]	Mean[1] r	% $P<0.05$	Range[1]	Mean[1] r	% $P<0.05$	Range[1]	Mean[1] r
Adult Males	5.7	0.10-0.16	0.13	4.4	0.07-0.11	0.08	7.9	0.04-0.08	0.06
Male Yearlings	7.0	0.11-0.28	0.17	11.6	0.06-0.18	0.09	14.1	0.04-0.12	0.06
Male Fawns	9.3	0.11-0.19	0.15	11.3	0.05-0.09	0.07	8.2	0.04-0.06	0.05
Adult Males and Female Yearlings	6.4	0.10-0.27	0.14	7.6	0.06-0.12	0.08	8.1	0.04-0.09	0.06
Adult Females	14.7	0.10-0.32	0.16	18.8	0.06-0.24	0.09	20.5	0.04-0.15	0.07
Female Yearlings	5.7	0.09-0.16	0.12	4.8	0.05-0.11	0.07	4.8	0.03-0.07	0.05
Female Fawns	17.1	0.10-0.24	0.14	15.2	0.06-0.13	0.09	19.5	0.04-0.09	0.06
Adult Females and Fawns	16	0.10-0.31	0.16	22.8	0.05-0.23	0.10	24.5	0.04-0.19	0.08

[1] Including only significant local r values.

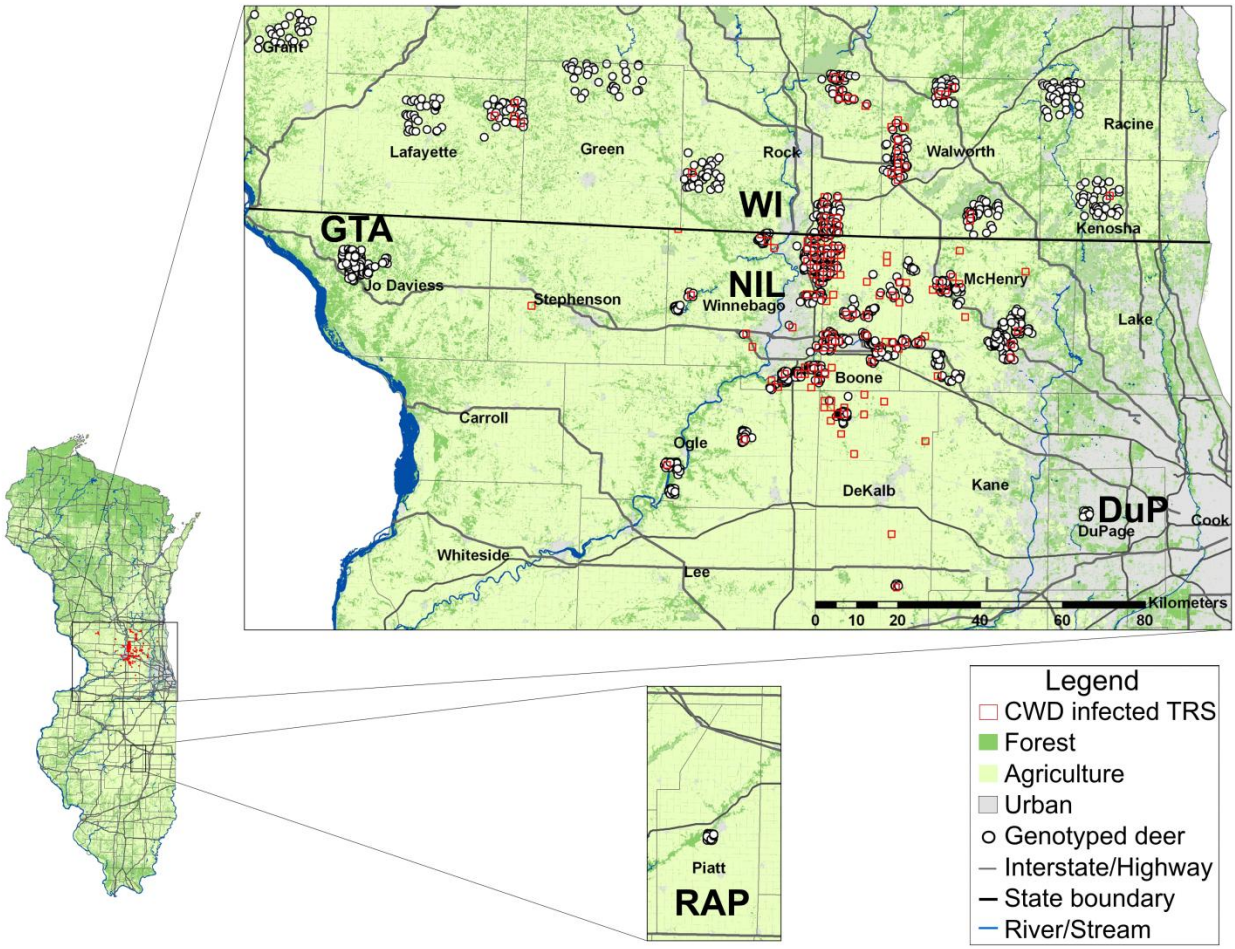

Figure 6.1. Wisconsin and Illinois sampling areas. WI=Wisconsin, NIL=northern Illinois, GTA=Galena Territories Association, DuP=DuPage Country Forest Preserve, RAP=Robert Allerton Park. Maps display the distribution of 1,988 white-tailed deer sampled for genetic analysis, where each open circle represents one genotyped deer and each open red box indicates a CWD infected TRS.

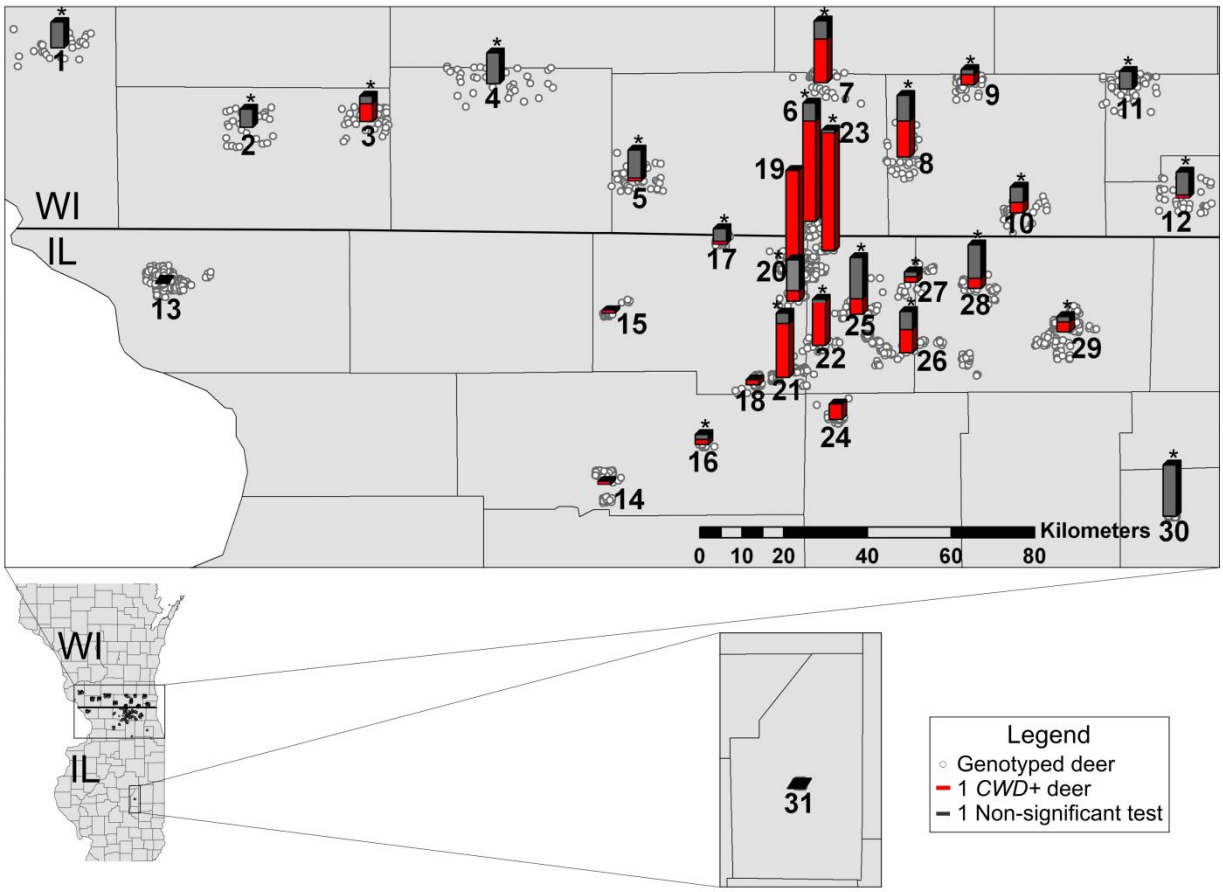

Figure 6.2. Genetic admixture of white-tailed deer in Illinois and southern Wisconsin. Chi-squared tests were conducted on allele frequencies of all deer among 31 study sites in Illinois and Wisconsin. The number of non-significant contingency tests for each study site was used as a metric for genetic admixture. The height of grey bars represents the number of non-significant contingency tests per study site. The height of red bars represents the number of CWD positive deer detected between 2003-2009 in each study site. Each open white circle represents a genotyped deer. Study sites with an * were genetically linked, and were considered samples from the same genetic population.

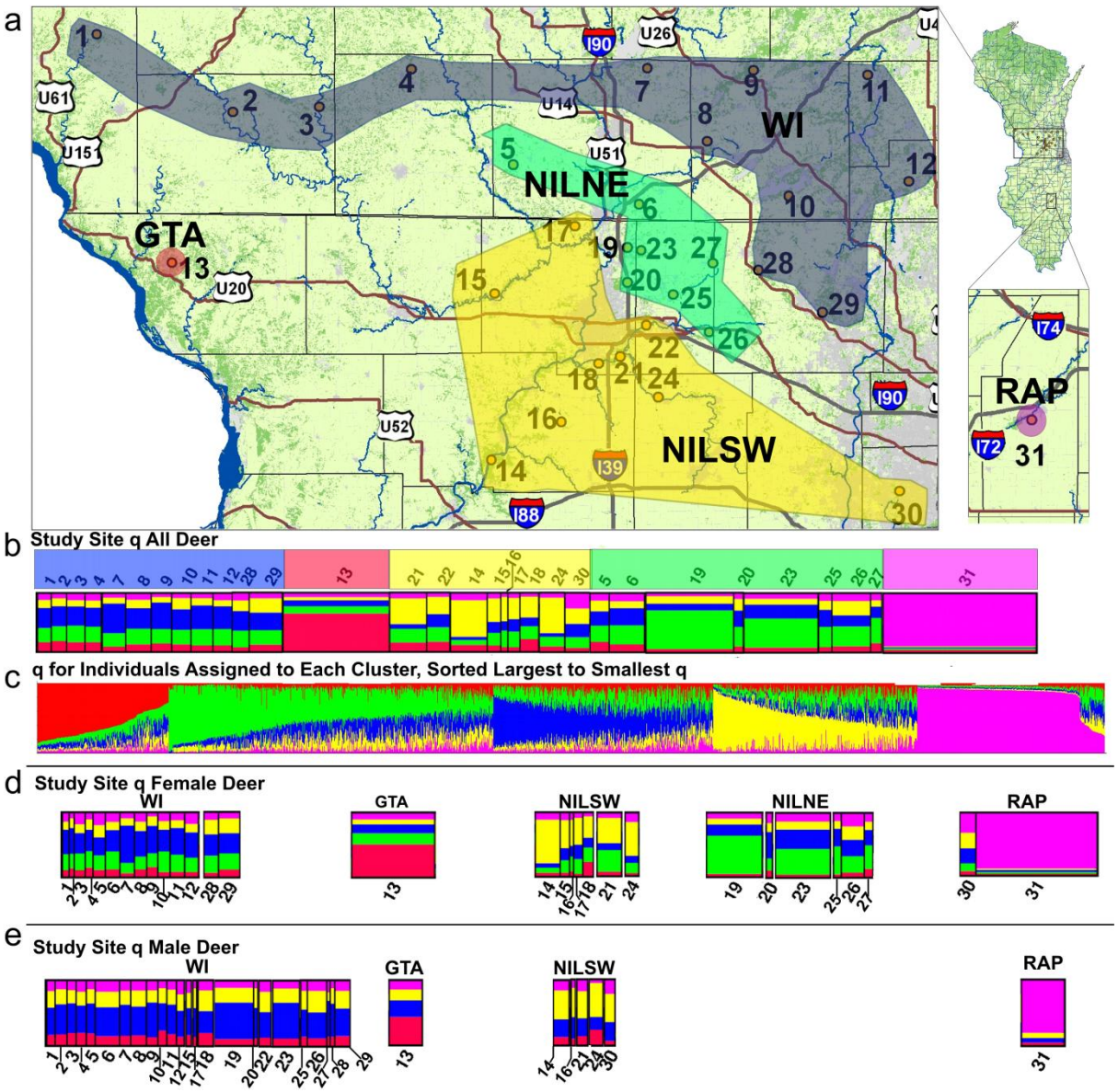

Figure 6.3. Genetic populations of deer in Illinois as revealed by Bayesian assignment of individuals. Clusters were simulated using STRUCTURE and study sites were assigned to the inferred cluster which contained the highest percentage of membership. Green represents membership assigned to NILNE, yellow represents membership assigned to NILSW, red represents membership assigned to GTA, blue represents membership assigned to WI, and pink represents membership assigned to RAP (panel a). Membership proportions (q) in the five inferred clusters are shown for all 31 study sites (panel b-all deer, panel d-female deer, panel e-male deer, sites separated by black vertical lines). Individual q values (panel c) were sorted from high to low for each inferred cluster to demonstrate admixture among adjacent populations.

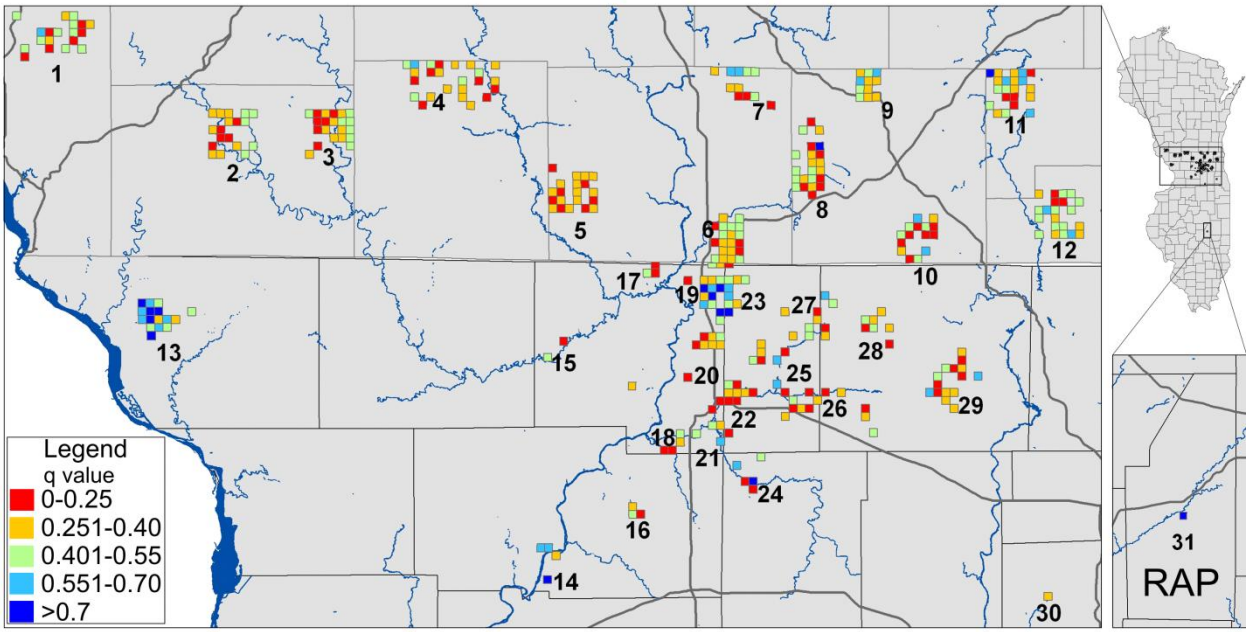

Figure 6.4. Average q values for white-tailed in Illinois and southern Wisconsin. Membership proportions (q) in the five inferred clusters were determined for individual deer using STRUCTURE and averaged for each TRS. Low q values are indicative of migrant ancestry while high q values are indicative of resident ancestry.

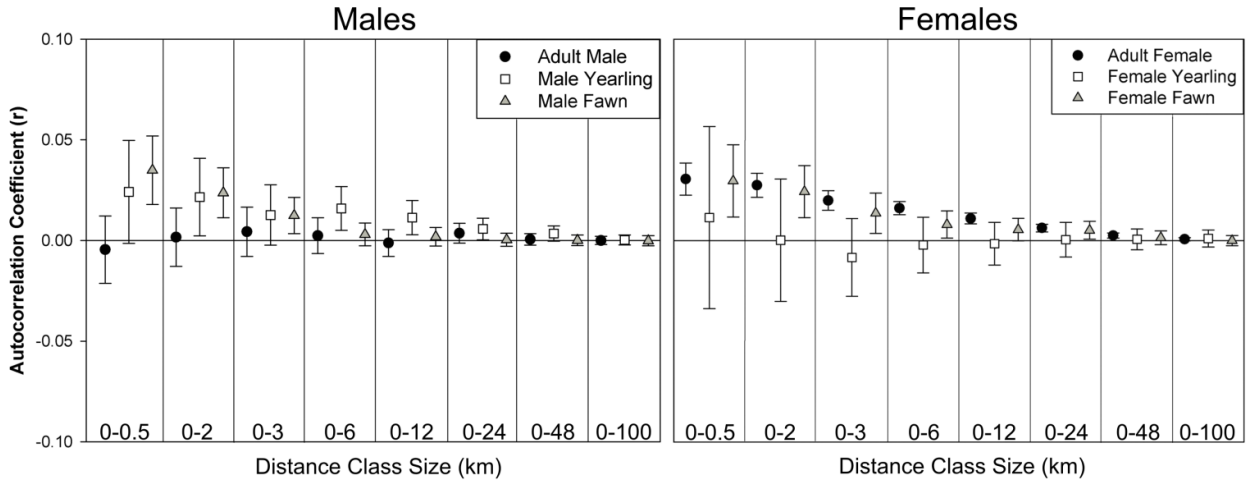

Figure 6.5. Global spatial autocorrelation for male and female deer grouped as fawn, yearling, and adult. Error bars surrounding the average *r* represent 95% confidence limits calculated with 999 bootstraps.

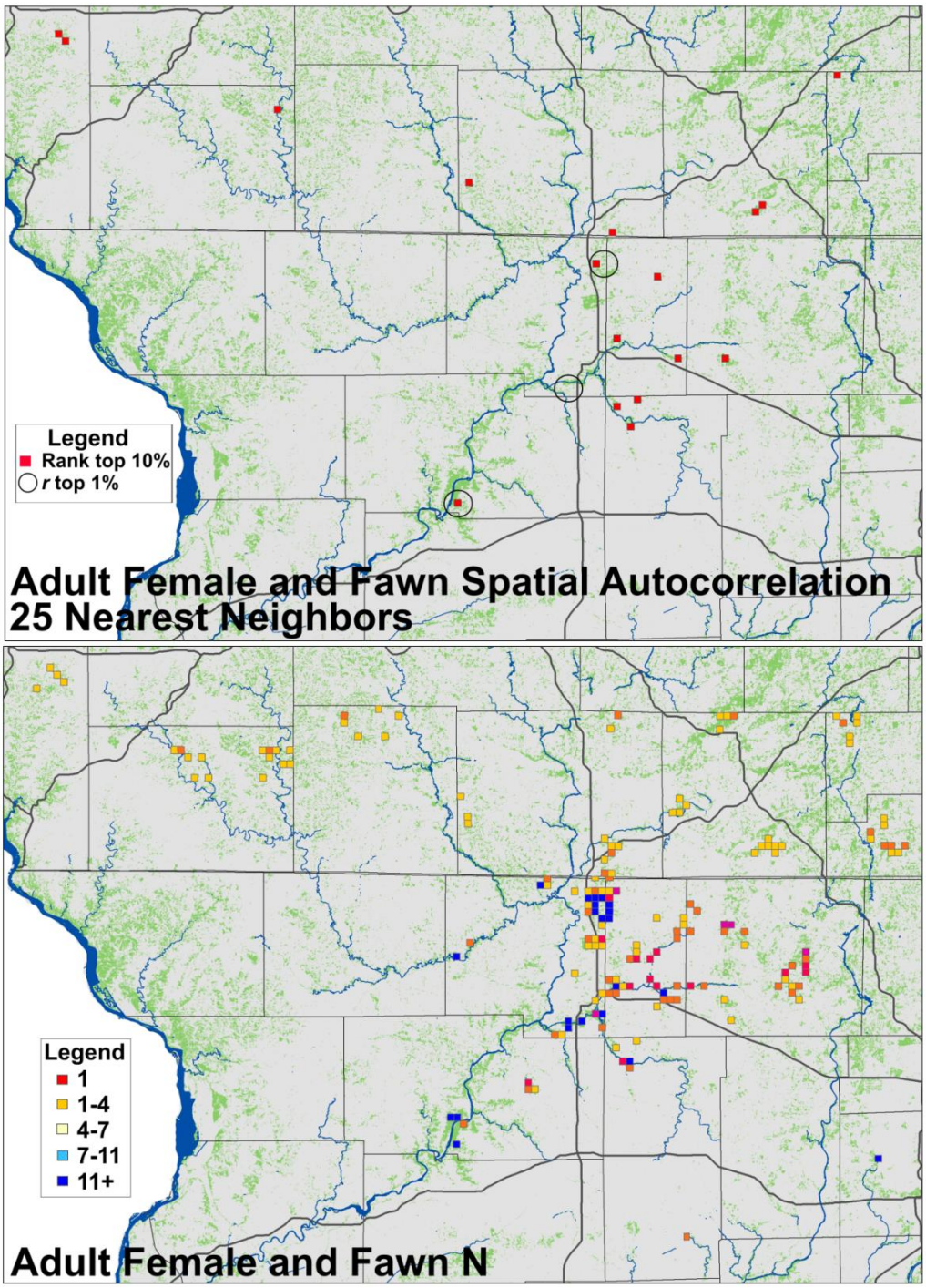

Figure 6.6. Local spatial autocorrelation with 25 nearest neighbors (top panel) and N (number of individuals sampled per TRS; bottom panel) for adult females and fawns of both sexes. Two dimensional local spatial autocorrelation analyses were conducted in GenAlEx using 999 conditional permutations to assess the significance of *r*. To account for multiple samples within a single TRS, autocorrelation coefficients for adult females and fawns were ranked according to conditional significance (highest correlation=1). Rank values were then averaged for each TRS and the top 10% were mapped (top panel). The highest 1% of individual *r* values were also mapped to show areas where single observations indicated relatively high autocorrelation.

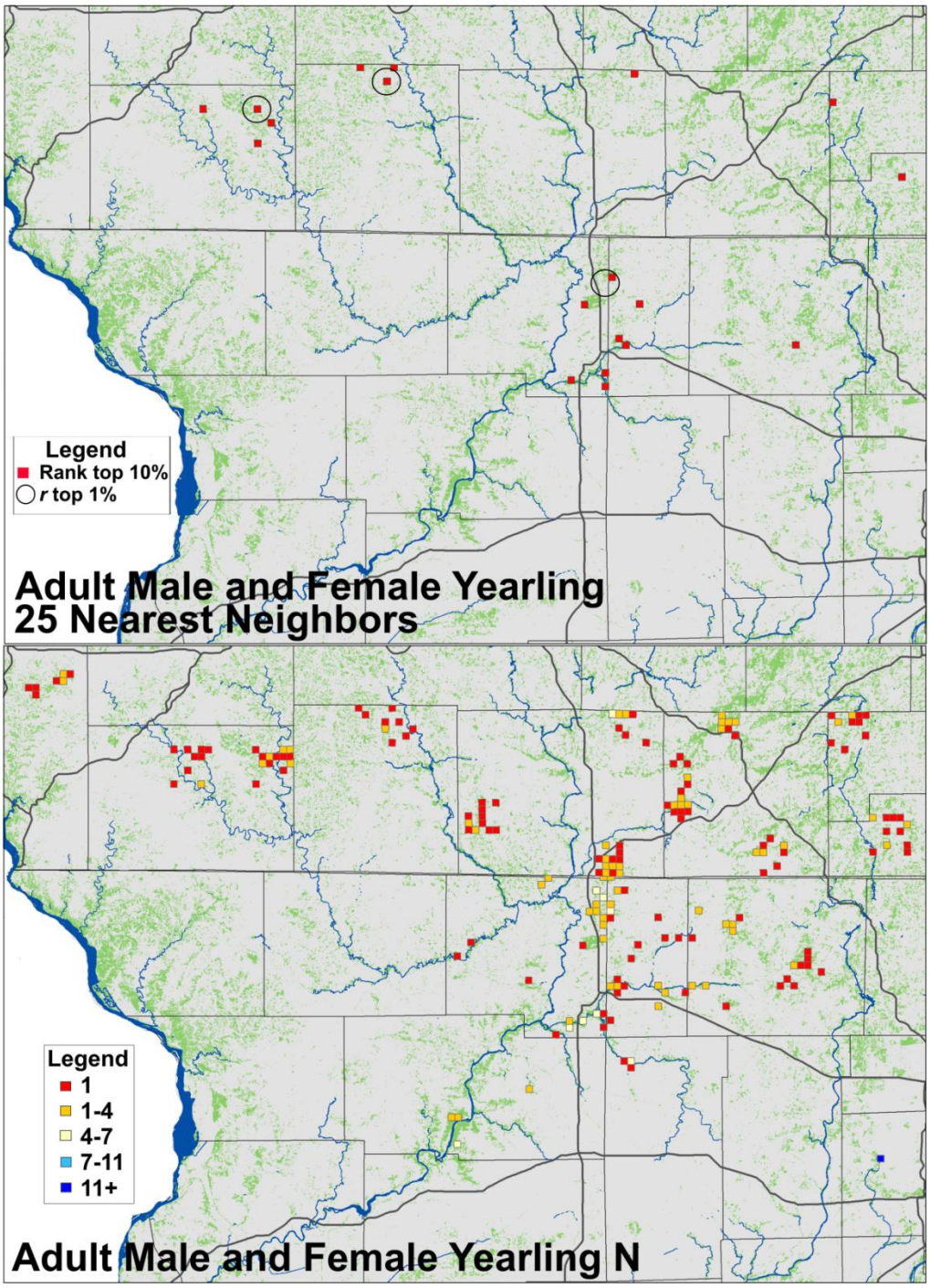

Figure 6.7. Local spatial autocorrelation with 25 nearest neighbors (top panel) and N (number of individuals sampled per TRS; bottom panel) for adult males and female yearlings. Two dimensional local spatial autocorrelation analyses were conducted in GenAlEx using 999 conditional permutations to assess the significance of *r*. To account for multiple samples within a single TRS, autocorrelation coefficients for adult males and yearlings were ranked according to conditional significance (highest correlation=1). Rank values were then averaged for each TRS and the top 10% were mapped (top panel). The highest 1% of individual *r* values were also mapped to show areas where single observations indicated relatively high autocorrelation.

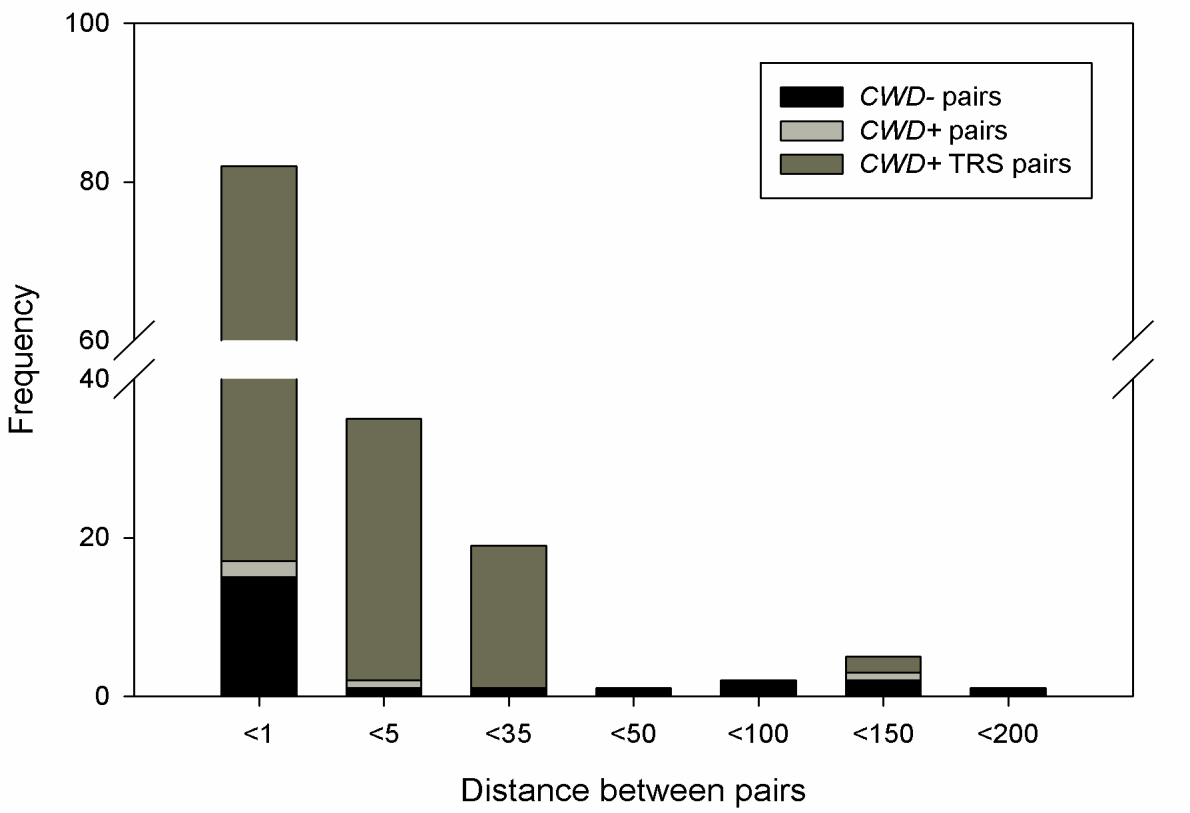

Figure 6.8. Histogram showing pairwise distances between CWD infected TRS and distances between mother-offspring pairs of white-tailed deer in WI, NIL, GTA and DuP. Using parentage analyses in Cervus, 27 mother-offspring pairs were identified at 95% confidence, with four of these pairs involving CWD-infected deer.

Chapter 7: CWD Resistance and Geographic Variation of Prion Gene Polymorphisms in Illinois White-tailed deer (*Odocoileus virginianus*)

Abstract

Chronic wasting disease is an infectious prion disease in cervids that is highly transmissible by direct contact and environmental exposure to disease agents. Certain genotypes of the prion gene (*Prnp*) have been shown to prolong disease progression and survival of CWD infected deer. Examining *Prnp* genotypes in CWD infected and uninfected deer populations can reveal associations between genotype and phenotype to determine if selective pressures are affecting *Prnp* allele frequencies. If selection is occurring, we would expect *Prnp* genotypes that prolong survival to be higher in infected populations compared to uninfected populations. To test this hypothesis, we genotyped 219 (99 CWD positive and 120 CWD negative) deer from the CWD outbreak region of northern Illinois and southern Wisconsin. We also sampled deer from two uninfected populations: one ~150 km away from the outbreak region, and another ~300 km from the outbreak region. Twelve nucleotide polymorphisms, eight silent and four coding, were found in the sampled populations. Five polymorphic loci had significantly different distributions of alleles between infected and uninfected individuals. Nucleotide base changes 60C/T, 285A/C, 286G/A, and 555C/T were observed with higher than expected frequencies in CWD negative animals suggesting disease resistance, while 153C/T was observed more than expected in positive animals, suggesting susceptibility. The total number of polymorphisms per animal, silent or coding, was negatively correlated to disease status. Polymorphisms 243T/A, 286G/A and 555C/T were found at higher than expected frequencies in uninfected populations. The total number of polymorphisms, both silent and coding, also differed between infected and uninfected populations. At the temporal scale examined, selection does not appear to be favoring genotypes

associated with CWD resistance as these genotypes tended to have higher frequencies in uninfected populations.

Introduction

Prion diseases, such as chronic wasting disease (CWD), scrapie and bovine spongiform encephalopathy (BSE), are caused by conversion of endogenous, host-encoded prion protein (PrP) to an abnormal disease causing conformation designated PrP^{sc} (Prusiner 2004). Though spontaneous disease occurs, most animals become infected following exposure to PrP^{sc} (Belay and Schonberger 2005). Chronic wasting disease is the only prion disease established in free ranging animals including white-tailed deer, mule deer, elk and moose. Factors related to CWD transmission are of particular interest because of its spread in the wild and ability to become established under natural population densities. Studies in captive cervids suggest CWD lateral transmission occurs similarly to scrapie in sheep (Williams and Young 1992; Miller and Williams 2003). Environmental transmission of CWD has been demonstrated in captive herd holding pens contaminated with carcass residue or fecal material (Miller and Williams 2003) and may also occur by infectious prions present in blood and saliva of deer with CWD (Mathiason *et al.* 2006). The first reported occurrences in wild deer and elk were found in Colorado and Wyoming in 1985. The first positive cases of CWD east of the Mississippi river were detected during 2002 in Wisconsin (Joly *et al.* 2003), and another outbreak was discovered in Illinois later that same year (Illinois Department of Natural Resources 2010).

Prion protein sequence is important in many aspects of prion disease including etiology, pathology, and transmission. A number of PrP polymorphisms that alter resistance to prion disease have been documented in many species (Novakofski *et al.* 2005) and several may alter CWD susceptibility in deer. For example, in mule deer the S225F polymorphism was strongly

related to CWD susceptibility, with SS225 deer 30 times more likely to be infected (Jewell *et al.* 2005). In white-tailed deer Q95H and G96S polymorphisms have been reported in Colorado (O'Rourke *et al.* 2004), south central Wisconsin (Johnson *et al.* 2003; Johnson *et al.* 2006), and northern Illinois (Kelly *et al.* 2008). Johnson *et al.* (2006) demonstrated that GG96 individuals (homozygous for glycine, the consensus alleles at residue 96), showed increased deposition of PrP^{SC} in the obex than did GS96 individuals (heterozygous for G96S). In free ranging white-tailed deer Edmunds (2008) reported an average survival time of 24.7 months for SS96 CWD infected deer compared to a survival time of less than nine months for all other infected individuals.

In this study, we examined the relationship between prion polymorphisms and CWD phenotype in free-ranging white-tailed. We also compared *Prnp* genotypes among infected and uninfected populations from Illinois. Comparisons between *Prnp* polymorphisms in infected and uninfected deer will provide a better assessment of the underlying mechanisms that link *Prnp* with CWD susceptibility and resistance.

Materials and Methods

Deer Sampling

We utilized tissue samples collected through CWD surveillance and population control programs targeting Illinois. The majority of samples were collected from the CWD outbreak region in northern Illinois. Additional samples from culled populations were included to examine *Prnp* in uninfected populations that were geographically distant to the CWD outbreak in Illinois. Harvest date, gender, age and spatial locations were collected for all samples. Samples of obex and retropharyngeal lymph nodes were tested using USDA approved immunohistochemical procedures to detect protease resistant PrP at the Illinois Department of Agriculture Diagnostic

Laboratories in Galesburg or Centralia and most positive samples were confirmed at the National Veterinary Services Laboratory.

North-central Illinois sampling area (NIL). Between January 2003 and March 2009, Illinois Department of Natural Resources (IDNR) harvested ~5,000 free-ranging deer in the CWD-infected region of northern Illinois. Of the samples collected, 219 from Winnebago, Boone, DeKalb, Ogle, and McHenry counties were used for genetic analysis. Samples from all CWD positive deer with frozen tissue available were used for DNA extraction, PCR and *Prnp* sequencing. Given the nature of sample collection during surveillance efforts, we could not do a perfect paired-case control design, though for each CWD positive case, negative controls were selected on the basis of age, sex and geographic location. We controlled for age estimated by dentition because of reported age dependency for CWD prevalence in Wisconsin (Joly *et al.* 2003). The prevalence of CWD has also been reported to be gender dependent (Miller and Conner 2005) so whenever possible, both female and male negative controls of the same age were selected for each CWD positive deer. Furthermore, with the geographic spread of new CWD cases over time and the fragmented habitat in the study area, we felt it was important to also control for geographic variation in the samples from the infected population. Deer harvest locations were known to the nearest section (1.6 x 1.6 km) and controls were selected on this basis. Whenever possible control animals were selected from the same harvest year as the CWD case.

North-western Illinois sampling area (GTA). Between January and February 2008, 50 free-ranging deer harvested through population control programs from Galena Territory Association in JoDaviess County were selected for *Prnp* genotyping. These samples provided an opportunity to analyze uninfected populations ~150 km to the west of the CWD outbreak in Illinois. We

selected samples in GTA to match the age and gender distribution of deer selected in NIL, though this was not always possible because of differences in temporal sampling.

East-central Illinois sampling area (RAP). During fall hunting seasons between 2005 and 2007, 50 free-ranging deer harvested at University of Illinois Robert Allerton Park (RAP) through their Deer Management and Research Program were selected for *Prnp* sequencing. Samples collected in RAP allowed us to examine an uninfected population ~300 km from the CWD outbreak in Illinois. As with GTA, to the best of our ability, we selected samples in RAP to match the age and gender distribution of deer selected in NIL.

PCR. The methods of Kelly *et al.* (2008) were used for PCR and *Prnp* sequence evaluation.

Statistical Analysis.

Logistic regression and chi-squared tests using an α level of 0.05 were performed with SAS for Windows, version 9.1 (SAS Institute Inc). We used chi-square tests to examine differences in allele frequencies among infected (NIL) and uninfected populations (GTA, RAP) for each polymorphic genotype. Chi-square tests were also performed on genotypes to check for differences between observed and expected frequencies in both CWD negative and positive animals. GTA and RAP were omitted from analyses involving *Prnp* genotype and CWD phenotype to prevent bias associated with the sample size differences between uninfected and infected deer. When needed, the Mantel-Haenzel Chi-square Test was employed to correct for low frequency genotypes. Deviations from Hardy-Weinburg Equilibrium (HWE) were tested using the program GenAlEx (ver 6; Peakall and Smouse 2006).

Additionally, logistic regression was used to determine the relationship between polymorphisms and disease status in NIL. Genotypes were compared to consensus genotypes in an additive model as discussed by North *et al.* (2005). In this method genotypes were classified

as 0 = homozygous for the consensus genotype, 1 = heterozygous, 2 = homozygous for the polymorphism (North *et al.* 2005).

To determine the cumulative effects of polymorphisms, the total number of polymorphic alleles per animal was summed across all loci and regressed against CWD status. Observed allele frequencies were higher than expected for locus 153, so coding for this variable was reversed for cumulative regression models (2 = homozygous for the consensus genotype, 1 = heterozygous, 0 = homozygous for the polymorphism). The total number of polymorphic alleles (0,1, 2, 3, 4, 5 or 6) was examined in one model, the number of coding polymorphic alleles (0,1, or 2) was examined in a second model, silent polymorphic alleles (0,1, 2, 3, 4, 5 or 6) were evaluated in a third model, and coding and silent polymorphic alleles were tested simultaneously in a fourth model. To be sure that the susceptibility conferred by locus 153 did not have excessive influence, models were also run with this polymorphism omitted.

Haplotypes were generated from unphased genotypes using Phase, version 2.1 (Stephens *et al.* 2001; Stephens and Donnelly 2003). This program was also used to test for linkage disequilibrium between genotypes, and random distribution of haplotype frequencies between cases and controls. We also used logistic regression and chi-square tests to determine the relationship between disease status and sex, age, and gender.

Results

Prnp sequences were determined for 319 deer, 99 CWD positives and 220 uninfected individuals. In 319 deer, 12 single nucleotide polymorphisms (SNP) were detected (Fig. 7.1), eight of which had been previously described (nucleotide 286G/A Raymond *et al.* 2000; nucleotides 60C/T, 153C/T, 438C/T, 555C/T Heaton *et al.* 2003; nucleotide 285A/C, Johnson *et*

al. 2003; nucleotides 243T/A, 438C/T O'Rourke *et al.* 2004; nucleotide 676C/A EMBL AY425673).

Blast and literature searching indicated that 308A/T, 367G/T, 372G/A and 378G/A have not been previously reported, consistent with our observation that these polymorphisms were at low frequencies (<2%) in sampled deer. Eight of these polymorphic loci were silent and translated to a synonymous amino acid sequence, while four of the SNP translated to a change in amino acid sequence of the protein; nucleotide 285A/C to amino acid Q95H, 286G/A to G96S, nucleotide 308A/T to amino acid N103I and nucleotide 367A/C to amino acid A123T. With the exception of 555C/T, all of the observed allele frequencies of polymorphic loci (Fig. 7.1) were less than 50% which indicated that the database derived consensus sequence was also the wild type genotype in Illinois white-tailed deer. In all deer sampled, the frequency of 555T was 72.4% indicating that this was the consensus allele for the population. However, while frequencies of 555T were higher than 555C in GTA and RAP, in NIL 57.3% of deer had 555C indicating that it was the consensus allele for this population.

Polymorphisms and CWD status in NIL. Five of twelve polymorphisms were significantly related to CWD status according to Chi-square tests (Table 7.1). Loci 60, 285, 286, and 555 had higher than expected frequencies of polymorphisms in CWD negative animals. The opposite was true at locus 153 where observed frequencies of polymorphic alleles were higher than expected in CWD positive animals. Logistic regression also indicated that polymorphisms at loci 60, 285, 286, and 555 were protective against CWD ($P < 0.05$) as genotypes at these loci had odds ratios less than one (Table 7.1). Individuals heterozygous for polymorphisms at 60, 285, 286 and 555 had significantly reduced odds ratios. Odds ratios for homozygous polymorphisms 60T/T and 285C/C overlapped one and were thus not considered significant. The low number of individuals

homozygous for polymorphisms at locus 286 prevented calculation of meaningful risk reduction for this genotype. Still, no CWD positive individuals were 286S/S, consistent with the observation that this genotype is protective against disease (Johnson *et al.* 2003; O'Rourke *et al.* 2004; Johnson *et al.* 2006; Edmunds 2008; Kelly *et al.* 2008). Individuals with a homozygous 555T/T genotype also had a reduced odds ratio of 0.37 for CWD (Table 7.1), though the odds ratio for heterozygous genotypes at this locus overlapped one and was not considered significant.

The polymorphism at locus 153 was a risk factor for CWD (Table 7.1), with the heterozygous 153C/T genotype almost doubling the risk of being CWD positive (odds ratio of 1.89). Although only four deer were found to be polymorphic homozygous at 153 (153T/T), this genotype produced a near six-fold increase in the odds of CWD. However, the odds ratio for heterozygous genotype 153C/T (Table 7.1), was not considered significant because it overlapped one.

In NIL, loci 243, 286 and 676 were found to significantly deviate from HWE ($P < 0.001$). The other nine loci did not deviate from HWE expected frequencies in NIL. Linkage disequilibrium between loci was observed in both positive and negative deer with the strongest association occurring between loci 60 and 285 ($\chi^2 = 161.4$, $P < 0.001$). Seventeen deer were polymorphic at either locus 60 or locus 285 and 21 deer had both polymorphisms. Including both correlated loci in a multiple logistic regression model resulted in adjusted odds ratios of 0.48 and 0.40 for heterozygous genotypes 60C/T or 285A/C respectively.

Haplotype frequencies were estimated from unphased genotypes and the ten most common haplotypes (frequencies greater than 1%) are reported in Table 7.2. These haplotypes were present in 98% of deer sampled. Thirty different haplotypes were reconstructed in the sampled population with four haplotypes being distinct to the CWD positive population and nine

of the haplotypes restricted to negative animals. The permutation test performed on the haplotypes determined that CWD positive and negative animals had significantly different distributions of haplotype frequencies ($P < 0.01$), suggesting that genetic variation between CWD positive and negative populations exceeds genetic variation within the two groups.

Logistic regression models exploring multiple polymorphisms and disease status revealed that the risk of CWD was reduced as the total number of polymorphisms increased (Fig. 7.2). If deer were grouped by the cumulative number of polymorphic alleles per animal, a significant Wald Chi-Square was observed for the model ($P < 0.01$). Reverse coding or omission of locus 153 from models did not alter outcome, as both models were significant and produced similar odds ratio estimates ($P < 0.01$).

To examine relative contributions of expressed and silent changes to CWD resistance, coding and silent polymorphisms were modeled separately for multivariate analysis. Single variable models produced odds ratios of 0.25 for each coding polymorphic allele, and odds ratios of 0.58 for each silent polymorphic allele (Fig. 7.2). Multivariate modeling of silent and coding polymorphisms resulted in significant Wald Chi-Square ($P < 0.05$) tests for both variables and significant Log Likelihood ratio tests ($P < 0.01$).

We found no relationships between CWD status and age or gender although the experimental design was not intended to test this question.

The spatial distribution of silent and coding polymorphisms associated with CWD are shown for each sampled TRS in Fig. 7.4 and Fig. 7.5. Frequencies of these polymorphisms were highly variable across the study area. There did not appear to be areas where these polymorphisms were particularly rare or abundant, though this study was not designed to detect differences at such a fine spatial scale. Further, sampling heterogeneity could have contributed to

the observed patterns. Given the variance of allele frequencies among TRS, additional sampling would be needed to achieve sufficient power to resolve differences in allele frequencies if they exist at this spatial scale.

Geographic variation in polymorphisms. The polymorphism 378G/A was only observed in NIL, while 308A/T was only observed in GTA and 367G/T and 372G/A were only observed in RAP. Besides alleles that were exclusive to one population, Chi-square tests revealed that polymorphisms 243A/T, 286G/A and 555C/T had significantly different genotype frequencies among the three sampled populations (Fig. 7.3). At locus 243, genotype frequencies in NIL and GTA were similar, but RAP had a much higher frequency of heterozygotes than did the other populations (10% in RAP compared to 2% in GTA and 1.83% in NIL). At locus 286, the consensus genotype was higher in NIL (79% in NIL, 62% in GTA and RAP) while the other two populations had higher frequencies of heterozygous genotypes at this locus (36% in GTA, 34% in RAP, 16% in NIL). Similarly at locus 555, frequencies of the consensus genotype, 555C/C were higher in NIL (33% in NIL, 12% in GTA, 20% in RAP), while RAP and GTA had higher frequencies of heterozygous genotypes at this locus (60% in RAP, 72% in GTA, 49% in NIL). All three populations had similar frequencies of 555T/T (range 16-20%). Loci 243, 286 and 676 deviated from HWE in NIL, but not in GTA or RAP. Locus 555 deviated from HWE in GTA ($P<0.01$), though none of the other loci deviated from HWE in GTA or RAP.

Haplotypes in GTA and RAP (frequencies greater than 1%) are reported in Table 7.2. As in NIL, linkage disequilibrium between loci 60C/T and 285A/C was observed in GTA and RAP ($\chi^2 = 227.9$, $P < 0.001$). The number of silent polymorphisms per animal was significantly different among the three study areas ($\chi^2=24.2$, $P<0.05$). NIL had a higher percentage of deer with zero, one, two and four silent polymorphisms than did GTA or RAP (Fig. 7.6). GTA had a

higher percentage of deer with three silent polymorphisms, and RAP had a higher percentage of deer with five and six silent polymorphisms. The number of coding polymorphisms per animal was also significantly different among the three study areas (χ^2=10.6, P<0.05). NIL had a higher percentage of deer with zero coding polymorphisms, RAP had a higher percentage of deer with one coding polymorphism and GTA had a slightly higher percentage of deer with two coding polymorphisms (Fig. 7.7).

Discussion

Polymorphisms that alter resistance to prion disease are present in many species (Novakofski *et al.* 2005). For example, in sheep PrP containing the V136 R154 Q171 polymorphic sequence confers scrapie susceptibility while a combination of A136 R154 R171 alleles confers scrapie resistance (Baylis and Goldmann 2004; Roden *et al.* 2006) and selection for the resistant alleles has been used in the European Union to reduce the prevalence of scrapie (Gubbins and Roden 2006). Wildlife managers are similarly interested in the potential of genetic resistance in managing CWD, both for understanding or predicting spread of disease, and for the possibility of use in repopulation following herd reductions. Furthermore, information about polymorphisms and susceptibility in additional species could contribute to the general understanding of prion disease mechanisms.

This study identified allelic variants at nucleotide positions 60, 285, 286, and 555 (codons 20, 95, 96 and 185) of white-tailed deer *Prnp* associated with resistance and at nucleotide 153 (codon 51) associated with susceptibility to CWD. The association with resistance to CWD of polymorphisms 285A/C which codes for Q95H and 286G/A which codes for G96S, confirms previous reports of a genetic trend or association in enclosed herds (O'Rourke *et al.* 2004) and in a free-ranging white-tailed deer population (Johnson *et al.* 2006; Kelly *et al.* 2008). It has been

reported that S96 (286G/A) transgenic mice inoculated with CWD displayed high levels of resistance to multiple strains of the disease when compared to their G96 transgenic counterparts (Meade-White *et al.* 2007), which is consistent with the absence of CWD positive deer homozygous for serine at amino acid 96 in the present study area. In contrast to our findings and studies in transgenic mice, Edmunds (2008) observed CWD infected free ranging white-tailed deer homozygous for serine at amino acid 96 in Wyoming, though these individuals had prolonged survival compared to GS96 deer and GG96 deer. Nonetheless, all of the aforementioned studies implicate G96S as a protective polymorphism in white-tailed deer.

Although we have detected *Prnp* polymorphisms that are associated with disease resistance, these alleles were not in higher frequencies in infected populations. Thirty-two percent of deer in Illinois had the consensus haplotype compared to 29% in both GTA and RAP, suggesting that *Prnp* sequence divergence in NIL is less than in the other populations. This was especially apparent for G96S where frequencies of heterozygotes were >2X higher in GTA and RAP compared to NIL (Fig. 7.3). This finding implies that natural selection has not favored this allele, or any of the other CWD associated polymorphisms, in infected populations.

In fact, strong deviations from HWE in NIL at locus 286 suggest selection against G96S. This is contradictory to the findings of Edmunds (2008), who suggested that increased survival of deer possessing G96S would result in higher frequencies in CWD infected populations. However, Edmunds (2008) examined deer from the CWD endemic area of Wyoming where CWD has been present for at least 25 years in wild deer herds (Spraker *et al.* 1997) at higher prevalence than in NIL (27% in WY compared to ≤10% in NIL) (Edmunds 2008). The recent discovery of CWD in NIL may have prevented the detection of selection pressures favoring G96S within the sampled timeframe. Further, Edmunds (2008) reported decreased survival

associated with CWD and currently, decreased survival in CWD infected versus uninfected deer has not been reported in Illinois. Therefore, it is possible that CWD is not a major source of mortality for NIL deer, in which case, strong selective pressures favoring CWD resistant alleles would not be expected.

Alternatively, locus 286 in *Prnp* may be linked to another nucleotide or gene that is under selection pressure. This has been observed for the doppel gene that is linked to bovine *Prnp* resulting in its apparent association with BSE in Fleckvieh cattle (Balbus *et al.* 2005). Linkage could also explain the association between silent polymorphism 60C/T and CWD. Polymorphism 60C/T was strongly linked to 285A/C which coded for a change in amino acid sequence. G96S could be linked to a gene that decreases survival of NIL deer, thereby creating selective pressures against this polymorphism in NIL. It has been suggested that migratory behavior in white-tailed deer has a genetic basis as it appears to be an adaptive trait (Nelson 1998). If *Prnp* genotypes were linked to such genes, they would be subjected to selection pressures acting on heritable behavioral traits. No such linkages have been identified to date, but further investigation into linkage effects may help explain our findings in NIL.

Chi-square tests revealed that polymorphisms 243A/T, 286G/A and 555C/T had significantly different genotype frequencies among the three sampled populations (Fig. 7.3). Further, 378G/A was only found in NIL, 308A/T was found only in GTA and 367G/T and 372G/A were only found in RAP. Differences in frequency were also reported for the two coding polymorphisms, Q95H and G96S, between central Wisconsin and NIL (Kelly *et al.* 2008). Collectively these findings imply that deer populations in Illinois and Wisconsin are genetically isolated to an extent. This is consistent with the findings of Kelly *et al.* (2010) who reported that GTA, NIL and RAP were separate populations according to allele frequencies of selectively

neutral markers. Genetic isolation may also imply that the central Wisconsin and Illinois CWD outbreaks occurred independently, thus offering some explanation for inconsistencies between geographically close outbreak sites.

The importance of polymorphism at amino acid 95 and possibly 96 in prion disease is likely related to metal binding which is in turn related to conformation, protease resistance and the ease of PrP conversion to PrP^{sc}. Numerous authors have suggested that metal binding in the proteolytic resistant portion of PrP, which extends from the octapeptide repeat region and includes residues 95 and 96, may modulate conformational change, self-association and PrP^{sc} propagation (Wells *et al.* 2006; Millhauser 2007). Protease resistance of the disease transmitting PrP^{sc} core, consisting roughly of PrP residues 90-230 (Safar *et al.* 1990), is a key component for oral and environmental transmission of prion disease since endogenous normal PrP is readily degraded by intracellular, digestive or microbial proteases, while PrP^{sc} is not.

At least one function of cellular PrP is intracellular transport of copper (Vassallo and Herms 2003). The N-terminal domain of the prion protein binds up to six Cu^{2+} ions. Copper binding occurs at histidine in four of the five octapeptide repeats as well as with the two histidine residues at amino acids 99 (H95 in mouse, H96 in human PrP) and 114 (H110 in mouse, H111 in human PrP). At higher copper concentrations, each Cu^{2+} ion is coordinated by a single histidine imidazole and an amide nitrogen from an adjacent glycine (Jackson *et al.* 2001; Burns *et al.* 2003). At lower copper concentrations, all six histidines are involved in binding two Cu^{2+} ions (Wells *et al.* 2006).

Mutations in *Prnp* linked to disease resistance in cattle and humans affect amino acid sequence of the copper binding portion of PrP (Owen *et al.* 1989; Beck *et al.* 2001; Capellari *et al.* 2002;Castilla *et al.* 2004). In cattle, insertions of octapeptide repeat regions within the copper

binding portion of PrP have been shown to increase rate of disease progression (Castilla *et al.* 2004). In humans, additional copies of octapeptide repeat regions within the copper binding portion of PrP are associated with familial Creutzfeldt-Jakob disease (CJD) (Owen *et al.* 1989).

Genetic mutations that enhance copper binding regions of PrP could make conversion into PrP^{sc} less efficient (Bocharova *et al.* 2005; Cox *et al.* 2006). It has been shown with recombinant PrP that increasing concentrations of Cu^{2+} decreases conversion of PrP to PrP^{sc}. As PrP binds additional molecules of copper, the flexible N-terminal of the protein is stabilized, which seems to prevent misfolding (Bocharova *et al.* 2005; Cox *et al.* 2006). This could explain why point mutations in deer producing additional histidine residues for copper binding are related to CWD resistance. Deer with polymorphic histidine at residue 95 of PrP could experience enhanced copper binding and therefore disease resistance. On the other hand, in humans, deletions of octapeptide repeat regions are associated with sporadic CJD 126. These deletions seemingly decrease copper binding ability in these individuals, thereby increasing their chances for sCJD (Beck *et al.* 2001; Capellari *et al.* 2002).

One explanation for these conflicting observations is that copper plays a different role in sequential steps of PrP^{sc} propagation. *In vitro*, copper attenuates conversion of PrP into PrP^{sc} fibrils (Bocharova *et al.* 2005) but also enhances protease resistance of PrP (Kuczius *et al.* 2004), as well as PrP^{sc} fibrils (Nishina *et al.* 2004; Bocharova *et al.* 2005). Though the mechanism remains unclear, copper binding would appear protective since Q95H animals were more likely to be CWD negative.

Synonymous or silent polymorphisms are non-coding and do not alter the amino acid sequence of translated protein, so it was initially surprising that they were significantly associated with CWD phenotype. However, insertions and deletions in the non-expressed

promoter region of *Prnp* in cattle and humans have been related to prion disease susceptibility (Sander *et al.* 2004; Haase *et al.* 2007). In cattle, insertions are located within binding sites for two different transcription factors and are associated with BSE resistance while deletions in this area are associated with BSE susceptibility (Sander *et al.* 2004; Juling *et al.* 2006; Haase *et al.* 2007). Insertions in the promoter region of bovine *Prnp* enhance transcription factor binding, which actually decreases *Prnp* expression levels through repressive influences (Sander *et al.* 2004). Decreased gene expression could lead to a decrease in the amount of intracellular PrP, thereby reducing the amount of substrate available for PrP^{sc} conversion (Juling *et al.* 2006). This hypothesis is consistent with findings in transgenic mouse models where over expression of host PrP significantly decreased survival time in mice inoculated with scrapie (Westaway *et al.* 1991), most likely because PrP conversion to PrP^{sc} was more efficient. Taken together, these findings suggest that alterations to non-coding regions of *Prnp* affect gene expression levels and therefore disease phenotype.

Consistent with the findings of Kelly *et al.* (2008), we observed a significant decrease in odds ratios for CWD as deer accumulated point mutations within *Prnp*. Mechanisms for the effects of multiple silent mutations on functional proteins have been described for other gene systems (Kimchi-Sarfaty *et al.* 2007). In humans, the multidrug resistance 1 gene (MDR1) codes for P-glycoprotein, a trans-membrane pump that moves certain drugs out of the cell. In this system, Kimchi-Sarfaty *et al.* (2007) found that an accumulation of silent single nucleotide polymorphisms (SNP) is accompanied by a decrease in substrate affinity for P-glycoprotein. Conformation-sensitive monoclonal antibodies were used to determine that conformational differences existed between wild-type and polymorphic P-glycoprotein despite identical amino acid sequences (Kimchi-Sarfaty *et al.* 2007).

Kimchi-Sarfaty *et al.* (2007) hypothesized that the structural differences between wild-type and polymorphic proteins were due to variations in post-translational folding resulting from alternative codon usage (Kimchi-Sarfaty *et al.* 2007). The idea that codons elicit variations in tertiary protein structure was introduced in 1987, by Purvis *et al.* (1987) who claimed that depending on the frequency of use, codons could alter protein translation rates and folding. For some proteins it appears that frequently used codons, such as those employed by wild-type alleles, facilitate rapid translation as compared to infrequently used codons that tend to delay translation (Purvis *et al.* 1987). In the case of MDR1, two of the synonymous mutations resulted in a switch from a frequent to an infrequent codon (Kimchi-Sarfaty *et al.* 2007). This change could have resulted in slower translation of P-glycoprotein which could alter its folding and therefore decrease substrate affinity. The inverse relationship between the number of point mutations within white-tailed deer *Prnp* and CWD status could be the result of a similar process. As silent mutations accumulate, chances of employing a rare codon increase, thereby providing the potential for alternate PrP translation and folding.

Ribonucleic acid (RNA) can also influence translational folding which may alter prion disease phenotype (Caughey and Kocisko 2003; Deleault *et al.* 2003). *In vitro* studies demonstrate that adding increasing doses of RNase to prion infected brain homogenate increasingly prevented conversion of PrP to PrP^{sc}. From this study it was concluded that host encoded RNA molecules are necessary cofactors for efficient PrP conversion to PrP^{sc} (Deleault *et al.* 2003). Another study used circular dichroism and recombinant sheep PrP to examine the effects of RNA on PrP conformation. Conversion of α-helix to β-sheet in recombinant sheep PrP was enhanced with the addition of wild-type sheep RNA (Liu *et al.* 2007). If the findings of Deleault *et al.* (2003) and Liu *et al.* (2007) are correct, then silent mutations within mRNA

transcripts of *Prnp* could be directly interacting with PrP during conversion, causing alterations in translational folding and affecting potential for PrP^{sc} formation (Caughey and Kocisko 2003). Further, RNA and DNA have been shown to bind with PrP and cause conformational changes resulting in protein aggregation (Nandi *et al.* 2002). It was suggested that this occurs because nucleic acids are acting as scaffolds that promote conversion of PrP to PrP^{sc} (Caughey and Kocisko 2003) and so silent mutations within DNA or RNA could alter nucleic acid-protein interactions, thereby decreasing the ease of conversion.

In general, it appears likely that most white-tailed deer residing in NIL have some degree of genetic susceptibility to CWD despite differences in genotypic frequencies between positive and negative animals. Further, comparisons with uninfected populations suggest that selection pressures associated with CWD mortality have not increased the frequency of resistant genotypes. The low frequency of G96S in NIL suggests that selection pressures are favoring the consensus genotype by mechanisms that are not understood. However, the deficiency of G96S in NIL is not necessarily harmful to the population as far as CWD is concerned. Increased survival (Edmunds 2008) and prolonged clinical progression (Johnson *et al.* 2006; Edmunds 2008) have been reported for infected individuals with this polymorphism, which could increase the duration of prion shedding (Edmunds 2008). Therefore, partial genetic resistance as observed in this study may prolong death of individuals, but it could also promote environmental contamination and therefore indirect transmission of CWD.

For managers, our findings suggest that stocking herds with genetically resistant deer is unlikely to fully prevent CWD, and it could lead to increased environmental deposition of infectious prions (Edmunds 2008). Further, there did not appear to be genetically isolated locales within NIL that harbored extremely high or low frequencies of CWD associated alleles, as

expected given the tendency for genetic admixture in this area (Kelly *et al.* 2010). This implies that sharpshooting for CWD management purposes is not targeting groups of deer that are highly resistant to CWD, though further research would be needed to fully quantify genetic resistance on microgeographic scales. Our research suggests strong associations between certain *Prnp* genotypes and CWD phenotype, but we did not consider environmental and demographic factors such as deer density or habitat composition that could affect disease occurrence and genetic exchange. It would be interesting to further investigate how these variables are related to genetic patterns of disease resistance and the epidemiology of CWD.

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Table 7.1. Ratio of observed and expected genotype frequencies for nucleotide polymorphisms in NIL.

Locus	Genotype	n	Frequency in Negatives	Frequency in Positives	Odds ratio	Wald 95% confidence interval
*60**	CC	185	78.33	92.08		
	CT	32	20.83	6.93	0.33	0.14-0.77
	TT	2	0.83	0.99		
*153**	CC	174	85.83	72.28		
	CT	40	13.33	23.76	1.89	1.04-3.43
	TT	5	0.83	3.96		
243	TT	214	97.50	98.02		
	TA	4	1.67	1.98		
	AA	1	0.83	0.00		
285*	AA	194	83.33	95.05		
	AC	**23**	**15.83**	**3.96**	**0.22**	**0.07-0.68**
	CC	**2**	**0.83**	**0.99**		
286*	GG	173	68.33	90.10		
	GA	**35**	**22.50**	**9.90**	**0.26**	**0.13-0.54**
	AA	**11**	**9.17**	**0.00**	**nc**	
308	**AA**	**219**	**100.00**	**100.00**		
	AT	**0**	**0.00**	**0.00**	**nc**	
	TT	**0**	**0.00**	**0.00**		
367	**GG**	**219**	**100.00**	**100.00**		
	GA	**0**	**0.00**	**0.00**	**nc**	
	AA	**0**	**0.00**	**0.00**		
372	GG	219	100.00	100.00		
	GA	0	0.00	0.00	nc	
	AA	0	0.00	0.00		
378	GG	218	99.54	100.00		
	GA	1	0.46	0.00	nc	
	AA	0	0.00	0.00		
438	CC	200	90.00	93.07		
	CT	19	10.00	6.93		
	TT	0	0.00	0.00		
*555**	CC	72	31.67	33.66		
	CT	107	44.17	55.45		
	TT	40	24.17	10.89	0.37	0.17-0.81
676	CC	215	96.67	100.00		
	CA	3	2.50	0.00	nc	
	AA	1	0.83	0.00		

* Indicates a significant association ($P < 0.05$) between each genotype and CWD status using Chi-square expected frequency. **Indicates $P < 0.05$ for parameter estimate in predictive models of CWD. Odds ratios were obtained from logistic regression models using each SNP as a class variable regressed against CWD as an outcome. Bold text indicates loci where nucleotide sequence resulted in a change of expressed protein. Grey shading indicates polymorphisms that were only detected in GTA or RAP.

Table 7.2. Reconstructed frequencies of common haplotypes for CWD positive and uninfected deer in NIL, RAP and GTA.

Haplotype Definition[1]	Frequency[2] in NIL (-) Deer	Frequency in NIL (+) Deer	Frequency in RAP	Frequency in GTA
CCTAGAGGGCCC	28	37	29	29
CCTAGAGGGCTC	26	34	25	32
CCTAAAGGGCTC	18	3	16	20
CTTAGAGGGCCC	7	15	10	6
TCTCGAGGGCCC	7	2	2	5
CCTAGAGGGTCC	5	3	3	3
TCTAGAGGGCCC	1	2	5	2
TCTAGAGGGCCA	2	0	0	0
CCTCGAGGGCCC	1	1	0	0
CCAAAAGGGCCC	1	0	0	0

[1]Haplotypes were generated from unphased genotypes with the Bayesian (ELB) algorithm in Phase ver 2.1. [2]Haplotype frequencies were significantly different between CWD positive and CWD negative animals ($P < 0.01$). Only haplotypes with a frequency >1% are reported.

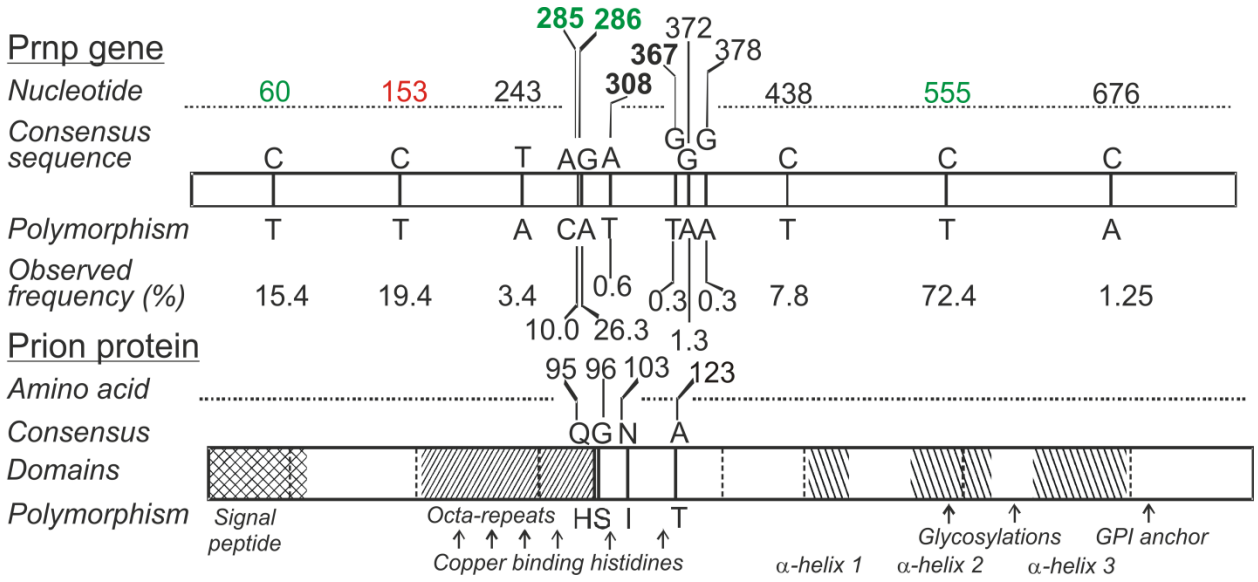

Figure 7.1. Nucleotide and amino acid database consensus sequence and polymorphisms observed in Illinois white-tailed deer. Numbers indicate the nucleotide or deduced amino acid sequence from a consensus. Bold indicates that the nucleotide substitution resulted in a change of amino acid sequence. Observed frequency (# of polymorphic alleles/total # of alleles*100) of polymorphisms and domains within the prion protein are also indicated. Nucleotides highlighted in green indicates polymorphisms that were observed in higher frequencies in CWD negative deer while the nucleotide highlighted in red indicates a polymorphism that was observed in higher frequencies in CWD positive deer.

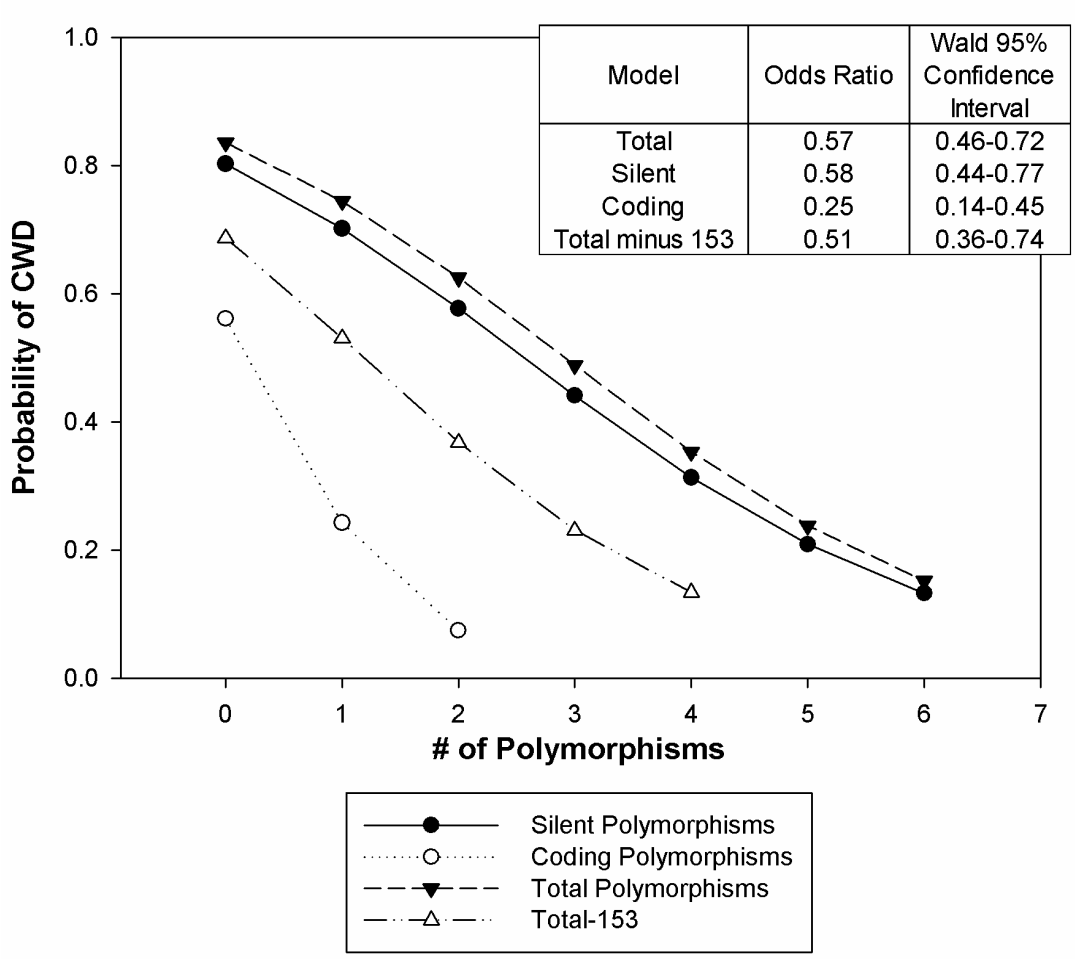

Figure 7.2. The total number of polymorphisms was used as a predictor of CWD in a logistic regression model. Odds ratios were estimated using the number of heterozygous and homozygous SNP as discrete values (0, 1, 2), summed across all loci.

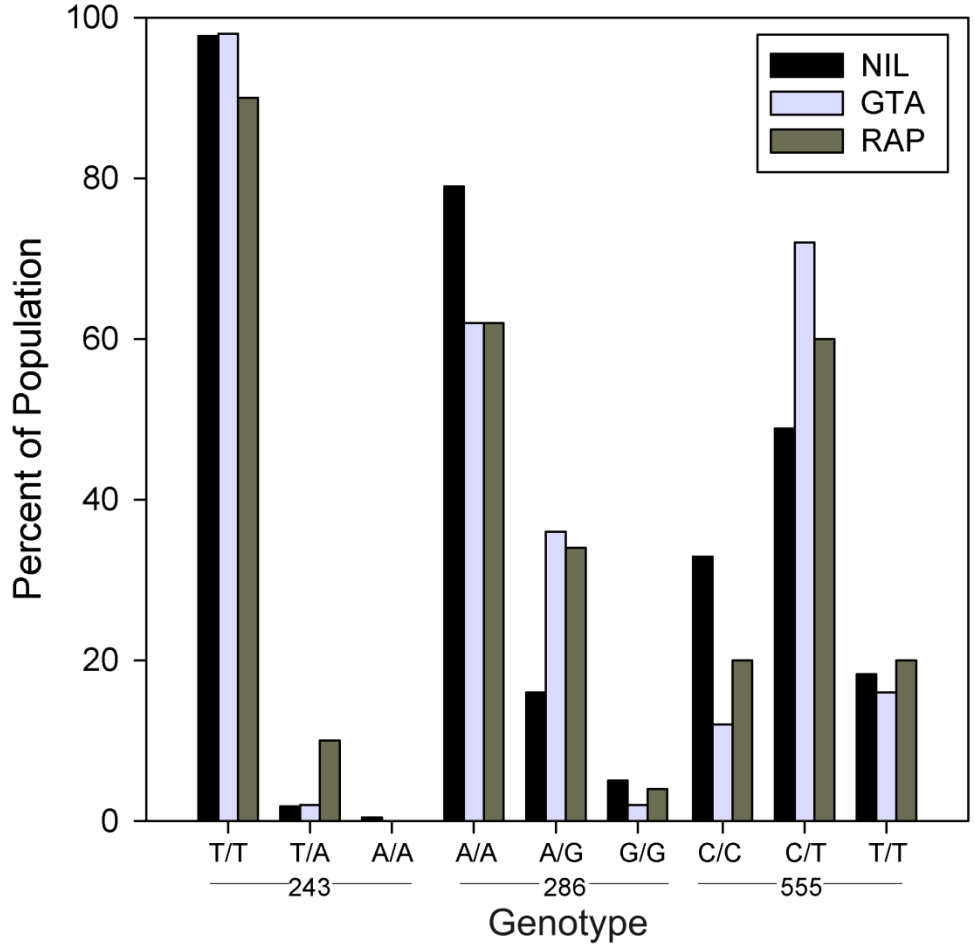

Figure 7.3. Distribution of polymorphisms at nucleotides 243, 286, and 555 in white-tailed deer from NIL, GTA and RAP. Bars represent the percent of sampled population of the indicated genotype.

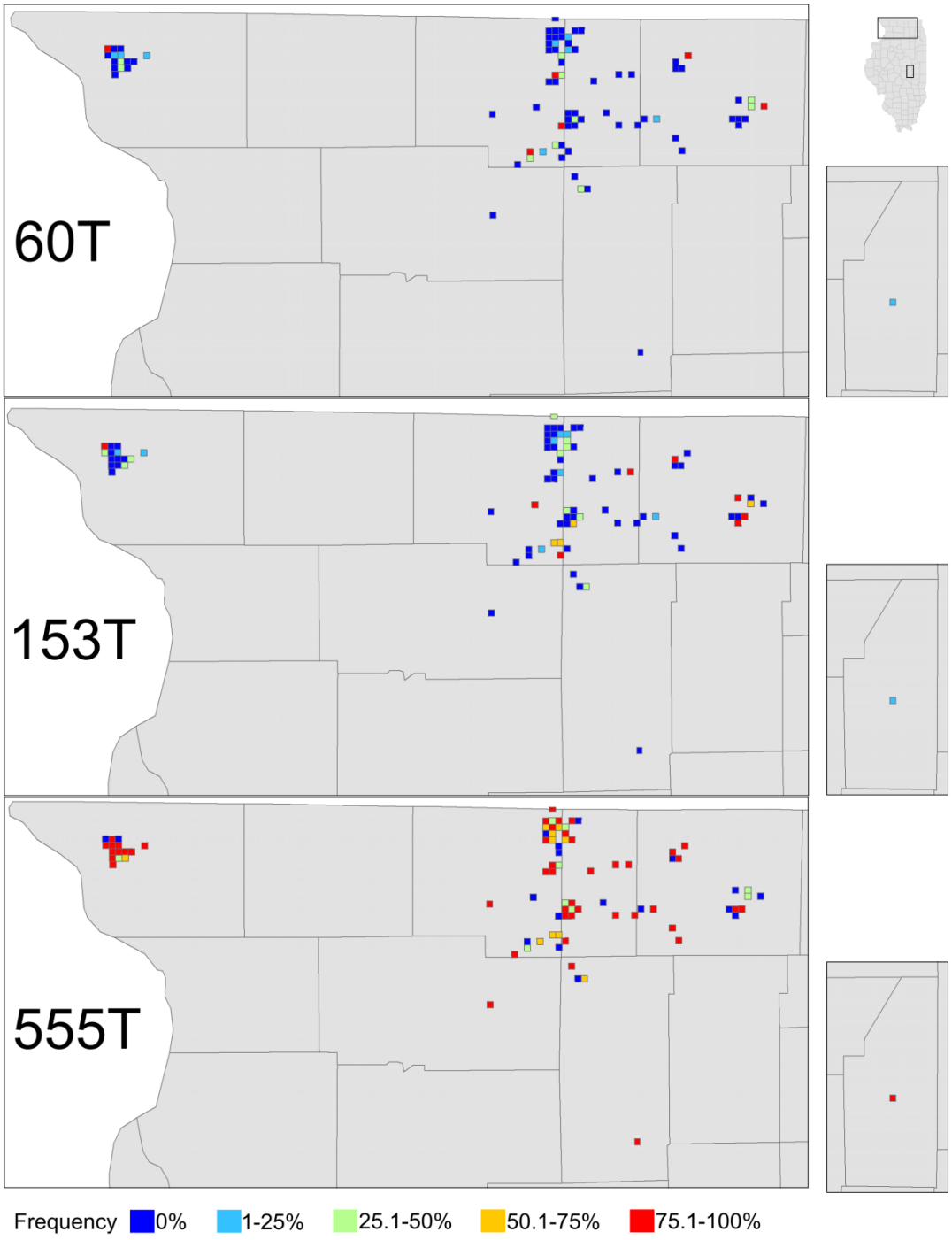

Figure 7.4. Spatial distribution of silent polymorphisms at nucleotides 60, 153 and 555 of *Prnp* in Illinois white-tailed deer. To calculate frequency, the number of individuals possessing each polymorphism was divided by the number of individuals sampled in each TRS and multiplied by 100. NIL and GTA are shown in the panels on the left while RAP is shown in the panels on the right. Polymorphisms at nucleotides 60 and 555 were associated with CWD resistance according to Chi-square expected frequencies, while polymorphism 153 was associated with CWD susceptibility.

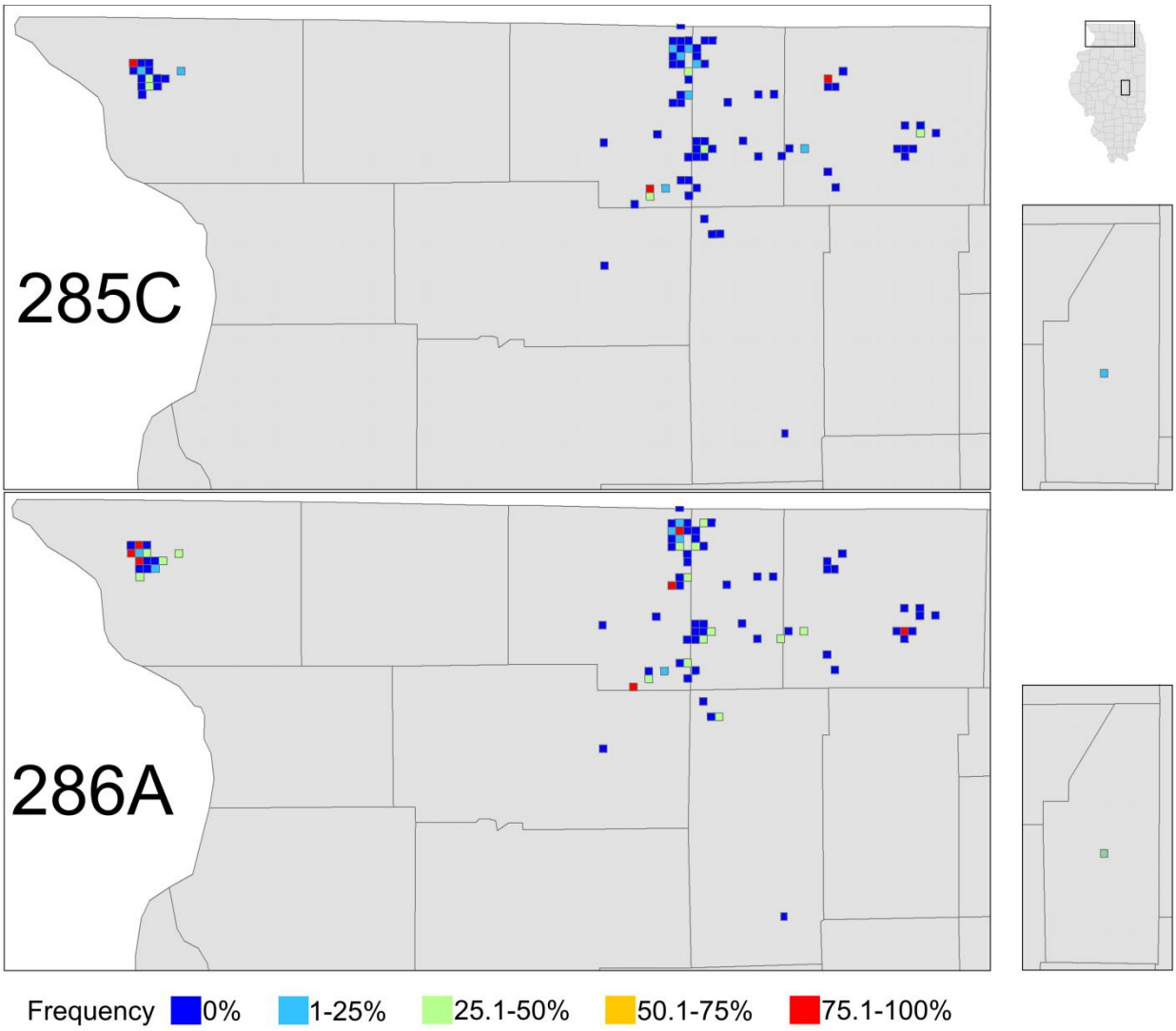

Figure 7.5. Spatial distribution of polymorphisms at nucleotides 285 and 286 of *Prnp* in Illinois white-tailed deer. To calculate frequency, the number of individuals possessing each polymorphism was divided by the number of individuals sampled in each TRS and multiplied by 100. NIL and GTA are shown in the panels on the left while RAP is shown in the panels on the right. The polymorphisms at 285 coded for a glutamine to histidine substitution at amino acid residue 95. Nucleotide 286 coded for a glycine to serine substitution at amino acid residue 96. While both polymorphisms were associated with CWD resistance, only 286G/A was found at higher frequencies in GTA and RAP than NIL.

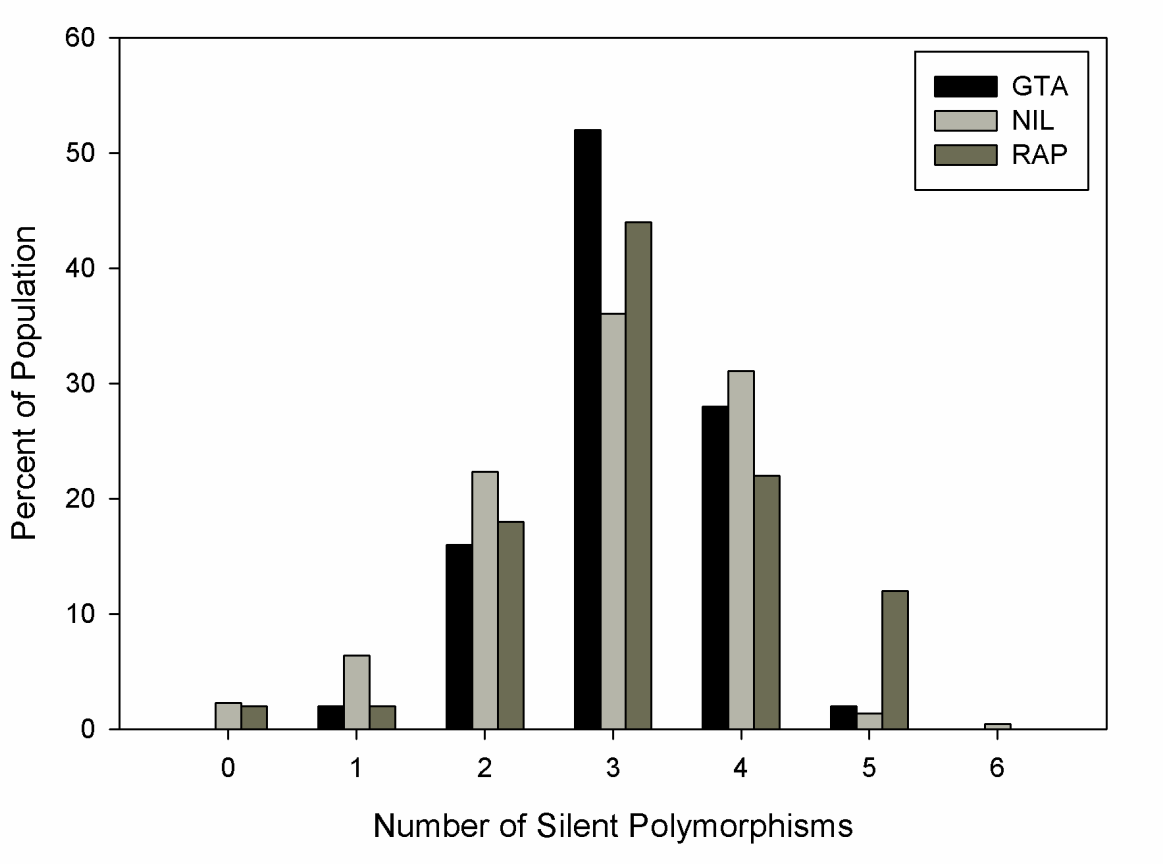

Figure 7.6. Distribution of silent polymorphisms in white-tailed deer from NIL, GTA and RAP. Bars represent the percent of sampled population of the indicated genotype. The total number of silent polymorphic alleles per animal was summed across all loci. Chi-square tests indicated that the distributions of silent polymorphisms differed among NIL, GTA and RAP (χ^2=24.2, P<0.05).

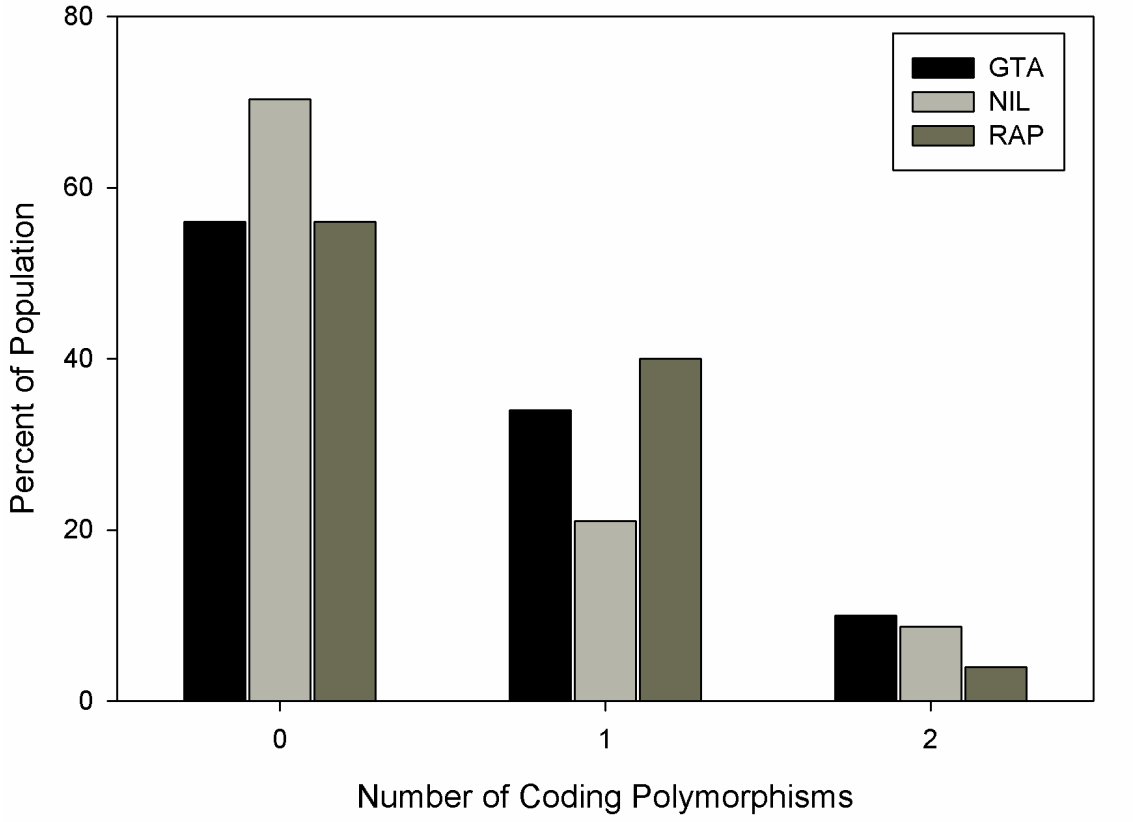

Figure 7.7. Frequency distribution of coding polymorphisms in white-tailed deer from NIL, GTA and RAP. Bars represent the percent of sampled population of the indicated genotype. The total number of coding polymorphic alleles per animal was summed across all loci. Chi-square tests indicated that the distributions of coding polymorphisms differed among NIL, GTA and RAP (χ^2=10.6, P<0.05).

Curriculum Vitae

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Education

- **Doctor of Philosophy**, Animal Sciences. University of Illinois, Urbana-Champaign. Dissertation: Landscape Genetics and Chronic Wasting Disease in White-tailed Deer. Graduate level course work in Statistics, Immunology, Geographic Information Systems, Epidemiology, and Ecology. April 2010.
- **Master of Science**, Animal Sciences. University of Illinois, Urbana-Champaign. Thesis: Prion Genetics of White-tailed Deer in Illinois. Graduate level course work in Genetics, Microbiology, Statistics, Animal Sciences and Biochemistry. August 2007.
- **Bachelor of Science**, Biology. University of Illinois, Urbana-Champaign. December 2004.

Professional Experience

- **Laboratory Experience:** Extensive experience in genetic fingerprinting, DNA fragment analysis, gene sequencing, DNA extraction, PCR, Western blotting and gel electrophoresis. Implementation of new protocols and laboratory techniques designed for processing thousands of genetic samples in an efficient high-throughput system.
- **Research Experience:** Research expertise utilizing applied and spatial statistics with novel applications to analyze molecular genetic data. Extensive experience adapting traditional statistical methods and population genetics techniques to study non-model organisms, specifically white-tailed deer in Illinois. Specialization in visual presentation of genetic data using a Geographic Information System to portray movement of genes across space and time.
- **Collaborative Skills:** Graduate research involves multidisciplinary projects within the Department of Animal Sciences, Department of Pathobiology, Illinois Natural History Survey, and Illinois Department of Natural Resources (IDNR). Extracurricular collaborative projects include examining reproductive biology of deer populations and investigating their physiological response to intense culling. Responsible for organizing Illinois State DNA archive for all samples collected from deer harvested by IDNR Chronic Wasting Disease surveillance program, 2002-2009.
- **Strong Communication Skills:** Extensive experience presenting results of graduate research to academia, industry and governmental agencies at scientific conferences, intellectual forums, workshops, and teaching academies. Presentation audiences have included funding agency representatives, animal scientists, molecular ecologists, wildlife biologists, graduate and undergraduate students, and the general public.
- **Additional Training:** Participated in several contracted FDA submission trials conducted at the University of Illinois Meat Science Lab. Investigated discovery-type compounds for numerous companies seeking improvements in livestock performance and production. Specific duties entailed

harvest and fabrication of animals, tissue composition analysis, data collection, report submission, and data entry/verification.

Leadership Experience & Extension

- **Teaching Assistant, University of Illinois:**
  - Microsatellite Analyses in Wildlife & Fisheries, Department of Integrative Biology, Spring 2009: Supervised multidisciplinary graduate level course covering laboratory and statistical techniques used in molecular genetics studies. Provided expertise on implementation and design of molecular markers for use in population genetic studies. Trained students to perform genetic fingerprinting in plants and animals, interacted with students to develop and optimize research protocols, and generated preliminary data for synergistic studies.
  - Wildlife Diseases Multidisciplinary Discussion Group, Department of Animal Sciences, Fall 2006-Spring 2007: Administrator for novel graduate level seminar series designed to familiarize students with epidemiology and management of wildlife diseases. Responsibilities included scheduling speakers for weekly lectures, coordinating discussion group topics, and facilitating interactions between faculty, undergraduates, and graduate students.
  - Animal Growth and Development, Department of Animal Sciences, Fall 2007: Undergraduate and graduate student course covering molecular and cellular determinants of tissue development and whole animal growth. Involvement included giving guest lectures, answering questions for students in class and during office hours, and holding review sessions to prepare students for exams.
- **Scientific Leadership:**
  - Four years experience training numerous undergraduates, graduate students, and scientific professionals in molecular and basic laboratory procedures including but not limited to DNA extraction, PCR and gel electrophoresis.
  - Voluntarily provided consultation for graduate students wishing to design and implement molecular genetics techniques in their own research. Adapted protocols developed in livestock for use in wild ungulates, endangered squirrels, plants, and insects.
- **Extension Work:**
  - Educational guide for Explore ACES, March 2005-March 2009. Activities included leading educational group tours of the Animal Sciences Facilities at the University of Illinois and tending educational exhibits for prospective students in the College of Agricultural, Consumer, and Environmental Sciences.
  - Member of IL Chapter of the Wildlife Society 2008-2009.
  - Member of the Pre-Veterinary Medicine Club 2000-2001.

Honors and Research Awards

- Invited attendee National Institute of Health National Graduate Student Research Fair, 2009.
- Best Student Presentation Award, Illinois Chapter of the Wildlife Society, 2009.
- Best Student Presentation Award, Illinois Chapter of the Wildlife Society, 2008.

- Howard Hughes Research Fellowship May 2001-June 2002.
  - Conducted research project exploring tetracycline resistance genes in *E. coli* samples from dairy farms across Illinois.
  - Learned to handle microorganisms, streak plates, isolate colonies of bacteria for DNA extraction, perform multiplex PCR, and gel electrophoresis.
  - Participated in a live animal study investigating the effects of TGF-Beta on bone regeneration. Used laboratory animals to evaluate the potential for TGF-Beta to treat human childhood cancers.
  - Monitored health status and administered injections to laboratory animals on trial.
- Dean's List 2000-2002.

Publications

1. **Amy Kelly**, Nohra Mateus-Pinilla, Marlis Douglas, Michael Douglas, William Brown, Marilyn Ruiz, John Killefer, Paul Shelton, Tom Beissel, Jan Novakofski. Utilizing CWD Surveillance to Examine Gene Flow and Dispersal in White-tailed Deer. *Journal of Applied Ecology*. 2010; Accepted.

2. **Amy Kelly,** Nohra Mateus-Pinilla, Marilyn Ruiz, Tom Beissel, Paul Shelton, Marlis Douglas, Michael Douglas, Jan Novakofski. Null Alleles Behaving Badly: Empirical Evaluation of Genotyping Errors and their Impacts on Population Studies. *Journal of Heredity*. 2010; Under Review.

3. **Amy Kelly**, Nohra Mateus-Pinilla, Emily Jewell, Tom Beissel, Jay Diffendorfer, John Killefer, Jan Novakofski, Paul Shelton. Prion Sequence Polymorphisms and Chronic Wasting Disease Resistance in Illinois White-tailed Deer (Odocoileus virginianus). *Prion*. 2008; 2:1-9.

4. **Amy Kelly**, Nohra Mateus-Pinilla, Marlis Douglas, Michael Douglas, Marilyn Ruiz, Tom Beissel, Paul Shelton, Jan Novakofski. Landscape Genetics of White-tailed Deer in Illinois: a Tool for Investigating an Outbreak of Chronic Wasting Disease. *The FASEB Journal.* 2009*;* 23: 926.9.

5. **Amy Kelly**, Nohra Mateus-Pinilla, Jay Diffendorfer, Michael Douglas, Marlis Douglas, Tom Beissel, Paul Shelton, John Killefer, Marilyn Ruiz, William Brown, Jan Novakofski. Resolution of Genetic Structure in White-tailed Deer: A Tool for Investigating an Outbreak of Chronic Wasting Disease in Illinois**.** *The FASEB Journal.*2008. 22; 173.4.

6. **Amy Kelly**, Nohra Mateus-Pinilla, Tom Beissel, Jay Diffendorfer, John Killefer, Jan Novakofski, Paul Shelton. Development of a Panel of Microsatellite Markers for the Assessment of Genetic Structure in White-tailed Deer in Northern Illinois and Southern Wisconsin**.** *The FASEB Journal*. 2007; 21: 120.11.

7. **Amy Kelly**, Emily Jewell, Nohra Mateus-Pinilla, Tom Beissel, Jay Diffendorfer, John Killefer, Jan Novakofski, Paul Shelton. Sequence Variation within the Prion Protein Gene from White-tailed Deer (*Odocoileus virginianus)* of Northern Illinois. *The FASEB Journal*. 2006; 20: A643.

Manuscripts in Preparation

1. **Amy Kelly,** Nohra Mateus-Pinilla, Marilyn Ruiz, Tom Beissel, Paul Shelton, Marlis Douglas, Michael Douglas, Jan Novakofski. Beyond State Borders: Landscape Genetics and CWD of White-tailed Deer from Illinois and Southern Wisconsin. *Molecular Ecology*. Manuscript in Preparation.

2. **Amy Kelly,** Nohra Mateus-Pinilla, Marilyn Ruiz, Tom Beissel, Paul Shelton, Marlis Douglas, Michael Douglas, Jan Novakofski. CWD Resistance and Geographic Variation of Prion Gene Polymorphisms in Illinois White-tailed Deer. *Journal of Wildlife Diseases*. Manuscript in Preparation.

3. **Amy Kelly,** Nohra Mateus-Pinilla, William Brown, Marilyn Ruiz, Tom Beissel, Paul Shelton, Marlis Douglas, Michael Douglas, Jan Novakofski. Effect of Landscape Features on White-tailed Deer Movement and Strucuture. *Molecular Ecology*. Manuscript in Preparation.

4. **Amy Kelly,** Nohra Mateus-Pinilla, Marilyn Ruiz, Tom Beissel, Paul Shelton, Marlis Douglas, Michael Douglas, Jan Novakofski. Genetic Characteristics of Managed White-tailed Deer Populations: Assessing the Impacts of CWD Surveillance. *Journal of Wildlife Management*. Manuscript in Preparation.

Abstracts

1. **Amy Kelly**, Nohra Mateus-Pinilla, Marlis Douglas, Michael Douglas, Marilyn Ruiz, Tom Beissel, Paul Shelton, Jan Novakofski. Using Molecular Genetics Techniques in White-tailed Deer: A Tool for Understanding Chronic Wasting Disease in Illinois.

2. **Amy Kelly**, Nohra Mateus-Pinilla, Jay Diffendorfer, Tom Beissel, Paul Shelton, John Killefer, Marilyn Ruiz, William Brown, Jan Novakofski. High-Throughput Analysis of Microsatellite Markers to Assess Genetic Structure in White-tailed Deer: A Tool for Investigating an Outbreak of Chronic Wasting Disease in Illinois.

3. **Amy Kelly**, Nohra Mateus-Pinilla, Jay Diffendorfer, Emily Jewell, Marilyn Ruiz, John Killefer, Paul Shelton, Tom Beissel, Jan Novakofski. Prion Sequence Polymorphisms and Chronic Wasting Disease Resistance in Illinois White-tailed Deer (Odocoileus virginianus).

Conference Presentations

Experimental Biology Conference, San Francisco, CA, 2006

- Sequence Variation within the Prion Protein Gene from White-tailed Deer (*Odocoileus virginianus)* of Northern Illinois (poster).

Conference of Research Workers in Animal Diseases, Chicago, IL 2006

- Sequence Variation within the Prion Protein Gene from White-tailed Deer (Odocoileus virginianus) of Northern Illinois (oral).

Experimental Biology Conference, Washington, D.C., 2007

- Development of a Microsatellite Marker Panel: Assessment of Genetic Structure in White-tailed Deer in Northern Illinois and Southern Wisconsin (oral).

Midwest Fish and Wildlife Conference, Madison, WI, 2007

- Prion Sequence Polymorphisms and Chronic Wasting Disease Resistance in Illinois White-tailed Deer (Odocoileus virginianus) (oral).

Wisconsin Chronic Wasting Disease Workshop, Madison, WI, 2006

- Polymorphisms within the Prion Gene from White-tailed Deer in Northern Illinois (oral).

Illinois Department of Natural Resources Chronic Wasting Disease Marksmanship Academy, Rock Falls, IL 2007

- Project Updates (oral).

Illinois Chapter of the Wildlife Society Annual Conference, Peoria, IL, 2008

- Resolution of Genetic Structure in White-Tailed Deer: Understanding Chronic Wasting Disease in Illinois (oral).

Experimental Biology Conference, San Diego, CA, 2008

- Resolution of Genetic Structure in White-Tailed Deer: Understanding Chronic Wasting Disease in Illinois (oral).

New and Re-Emerging Infectious Diseases, Urbana, IL, 2008

- Resolution of Genetic Structure in White-Tailed Deer: Understanding Chronic Wasting Disease in Illinois (poster).

Midwest Fish and Wildlife Conference Columbus, OH, 2008

- Resolution of Genetic Structure in White-tailed Deer (oral).

Illinois Chapter of the Wildlife Society Annual Conference, Sparta, IL, 2009

- Landscape Genetics of White-tailed Deer in Illinois: a Tool for Investigating an Outbreak of Chronic Wasting Disease (oral).

Experimental Biology Conference, New Orleans, LA, 2009

- Landscape Genetics of White-tailed Deer in Illinois: a Tool for Investigating an Outbreak of Chronic Wasting Disease (poster).

Midwest Fish and Wildlife Conference Springfield, IL, 2009

- Genetic Perspectives on White-tailed Deer Movement in Illinois (oral).